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Sun-Orientation and Homing in Fishes[1]

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ABSTRACT

The white bass, *Roccus chrysops*, has two principal spawning grounds 1.6 km apart on the northern shore of Lake Mendota. Fish displaced from these spawning grounds return faithfully to their respective spawning sites, and of 1366 fish marked and displaced to mid-lake, a distance of 2.4 km, 181 were recaptured in fyke nets and less than 9% erred by being recaptured at the net on the other spawning ground. Fish released at the spawning ground were not recaptured in greater percentage than those displaced and released in mid-lake.

Fish to which floats were attached for direct tracing moved generally north from the center of the lake when released on clear days. Moreover, fish released between the two spawning grounds also moved north on clear days. On cloudy days, however, or if blinded with eyecaps, they moved at random. Unexplained is how they differentiate between the two spawning areas; apparently it is by means other than with the aid of the sun.

A laboratory analysis of the sun-compass mechanism was made. An immature specimen of *Lepomis macrochirus* was trained, at a specific time of day, to find cover in one of sixteen boxes of a circular tank. When trained, the fish entered the training box in a consistent compass-direction at any time of day. Under an overcast sky the choices were completely unoriented. When tested under an artificial sun, (light bulb), this fish responded as though it were the real sun, at that time of day and sought cover in the "artificial" direction, reaffirming the presence of a biological clock. White bass were also successfully trained to a compass-direction under the natural sun.

Lepomis gibbosus was tested with another method. It too has a sun-compass mechanism.

These field and laboratory experiments suggest strongly that the sun serves as the point of reference, and that the animal compensates for its movement by a biological chronometer.

Fishes which migrate long distances, like the salmon and the eel, have various types of inherited and learned goal-finding mechanisms (Hasler 1956a). Some of these have been clarified, but many continue to puzzle us in our quest for an answer as to how fishes accomplish their spectacular homing feats. When such a fish is migrating toward home, over distances of several hundred kilometers, its ability to return would be facilitated if it had a compass and a chronometer as well as a sextant to aid in direction finding and in determining geographic location. With such mechanisms it could correct continuously to forces which drift it off course.

The various modes of homing orientation have been conveniently classified by Griffin (1952). They are modified here as follows:

Type I: The ability of an animal to find home by relying on local landmarks within familiar territory and the use of exploration in unfamiliar areas.

Type II: The ability to maintain a con-

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stant compass direction in unfamiliar territory. This type is called *Richtungsfinden (-fliegen)* by Kramer (1953).

Type III: The ability to head for home from unknown territory by true navigation. Designated by Kramer (1953) as *Zielfinden*. This involves a "sextant" type of mechanism, but as yet no observations have been made to describe its existence in fishes, although the birds appear to have one (Kramer 1952, and Sauer 1957).

It was our objective to study homing-behavior in less complex situations where the fish move shorter distances and are available for experimentation at appropriate times in their life cycle. Our observations were concerned with a description of homing in a large freshwater lake, and with evidence that orientation involving the use of a sun-compass could play a role in homing behavior. Information about geographic location is not essential over these distances; a compass, however, would be particularly useful to the fish to enable it to set a course in a given direction. With a sun-compass mechanism, which has already been established for other vertebrate and invertebrate animals (Kramer 1950 and 1952, von Frisch 1950, Pardi and Papi 1952), the fish could arrive in the vicinity of home. Once there, familiarity with combinations of visual, tactile, and olfactory features could direct it more precisely. In this regard Hasler and Wisby (1958) have evidence that largemouth bass (*Micropterus salmoides* Lac.) and green sunfish (*Lepomis cyanellus* Raf.) use local sign posts as points of visual reference in locating food, nest, or home territory. In the laboratory, as well, the Elritze (*Phoxinus laevis*) appears to use local visual marks in orientation to a feeding site (Hasler 1956b). This would be Type I, homing orientation of Griffin (1952).

HOMING IN WHITE BASS

For a number of years we have studied the natural history of the white bass (*Roccus chrysops* Raf.) in Lake Mendota, and we have been able to locate only two major spawning sites in the entire lake and these of very limited area. These spawning grounds, Maple Bluff and Governor's Island, are both on the north shore of the lake and are separated by a distance of 1.6 km (Fig. 1). Here the white bass congregate at spawning time in late May and early June at temperature ranges of from 16° to 24°C.

During three different spawning seasons

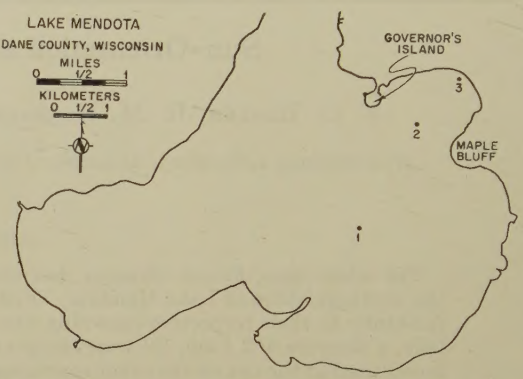

FIG. 1. Lake Mendota. Releases of white bass were made at Stations 1, 2, and 3.

TABLE 1. *Recapture data from 1,598 tagged white bass released in 1957 from A) a mid-lake point, and B) points on the Maple Bluff and Governor's Island spawning grounds of Lake Mendota*

Fish from spawning area:	No. tagged	No. re-captured	Per cent recaptured	No. re-captured at other spawning ground	Per cent correct return
A. *Releases from a mid-lake point*					
Maple Bluff					
♂	849	126	14.8	14	88.9
♀	100	4	4.0	0	100.0
Total	949	130	13.7	14	89.2
Governor's Island					
♂	343	50	14.6	2	96.0
♀	74	1	1.4	0	100.0
Total	417	51	12.2	2	96.1
B. *Releases from points on the spawning grounds*					
Maple Bluff					
♂	144	22	15.3	5	77.3
♀	19	0	00.0	0	—
Total	163	22	13.5	5	77.3
Governor's Island					
♂	41	4	9.8	0	100.0
♀	28	1	3.6	1	00.0
Total	69	5	7.2	1	80.0

(1955, 1956, 1957) white bass have been captured in fyke nets, marked with numbered Petersen tags, and transported in open tanks to the different release stations in the lake for daytime-releases. The fyke nets were emptied daily, weather permitting. All of the recaptures were recorded and a sample of new fish tagged before liberation.

From the start, we observed that a large percentage of displaced fish returned to the nets; moreover, as the observations accumulated, we were impressed with the precision of homing among the Maple Bluff and Governor's Island spawners. Table 1 gives convincing evidence of the precise ability of the white bass to return to their original spawning-site from a release-point located 2.4 km from the spawning grounds in a lake having an area 39.4 km^2 and with a shoreline of 32.4 km. The fyke nets were placed 15 m out from the shoreline and were connected to it by a lead. A white bass moving along shore near the bottom tends to be directed into the throat of the net; however, it may swim over or around it and thus avoid capture. A fish returning to the area of the net may therefore not be caught immediately because of the relative inefficiency of the recapturing trap. In fact, tagged fish when released on the spawning ground without being displaced, gave the same order of magnitude of recapture-percentage and time-lapse between release and recapture as did the fish that were displaced (Table 1, Fig. 2). The recapture-percentage and the per cent of correct returns of these two types of releases (Table 1) failed to show a real difference when tested statistically for significance. Therefore, this would indicate that there could have been an almost complete return of the displaced fish to a spawning ground, assuming similar mortality of the two groups, constant efficiency of the net and that the non-displaced fish remained as a catchable portion of the fish on the spawning grounds.

The differences in the recapture-percentage between the males and females (Table 1) probably reflects a difference in behavior at the spawning time: the ripe females move onto a spawning ground and remain only a short period until spawned, whereas the males stay in the area for a considerable time.

The number of fish returning and the accuracy of their return strongly support the view that the fish reach home with good precision. The nature of the method of recapture, however, does not lend itself to supplying information on the rapidity of return of the displaced fish. Therefore, from the tagging studies on white bass random searching cannot be discounted as a possible mechanism of return, but if this is

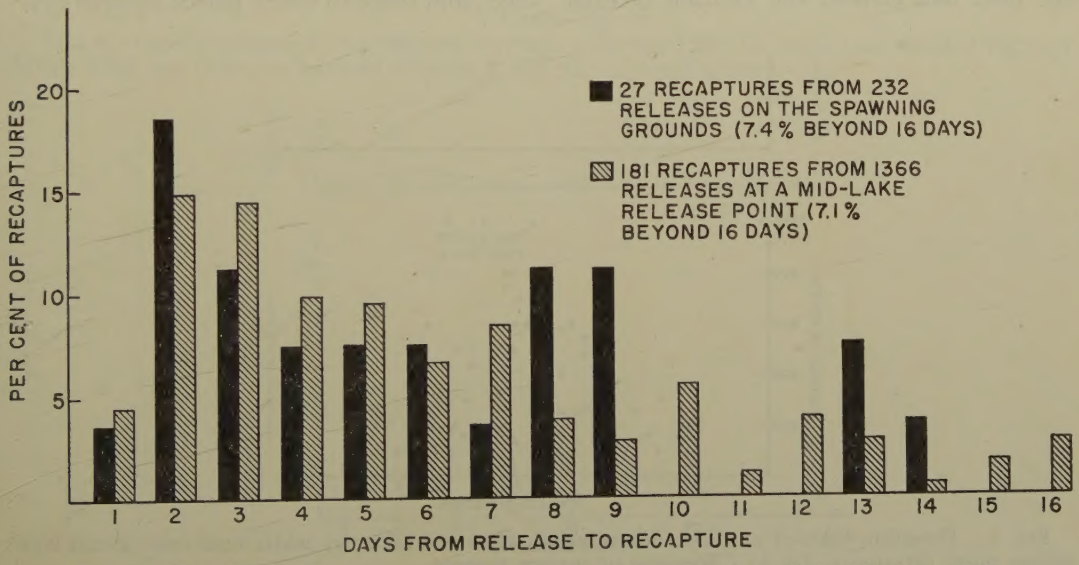
PER CENT OF RECAPTURES
20
15
10
5
27 RECAPTURES FROM 232 RELEASES ON THE SPAWNING GROUNDS (7.4 % BEYOND 16 DAYS)
181 RECAPTURES FROM 1366 RELEASES AT A MID-LAKE RELEASE POINT (7.1 % BEYOND 16 DAYS)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
DAYS FROM RELEASE TO RECAPTURE

FIG. 2. Time interval between marking and recapture—white bass, Lake Mendota, 1957.

the mechanism of return, then it is certain that these fish can distinguish accurately between the Governor's Island and Maple Bluff spawning grounds. It does not disprove, on the other hand, that clear-cut orientation to the spawning site (and direct movement) was involved in the return.

DIRECT TRACING OF DISPLACED FISH

The above data prove that the fish get back to their specific spawning ground, but they do not give us the information we need in order to know if the return was prompt and in a fairly direct path. An indicator of the direction of orientation at the release site was therefore needed—one which would compare with the observations of the "take-off-direction," a procedure which has advanced the knowledge of orientation in birds (Kramer *et al.* 1956). The technique described by Hasler and Wisby (1958) was used to establish "take-off-direction" of the white bass. Sexually ripe fish were removed from the fyke nets and transported quickly to the center of the lake. A fine monofilament nylon line (2–5 m in length) was fastened to the dorsal flesh, posterior to the dorsal fin, with a small fish-hook; this line was in turn attached to a yellow fisherman's bobber (64 mm in diameter). The individuals were released about 3 minutes apart until four or five had been liberated. After one hour had elapsed the location of each fish was established and charted. Figure 3 shows that the fish which were liberated on clear days in midlake (release station No. 1 in Fig. 1) moved generally north, toward the spawning grounds. For these releases the fish's final position was corrected for drift-displacement (Fig. 4), because the water currents are fairly homogeneous and directional over the distance the fish moved and could, therefore, favor or oppose its movement. However, to confirm this north-tendency, releases were also made between Maple Bluff and Governor's Island (release station No. 2 in Fig. 1). These fish also swam in a northerly direction (Fig. 5). Here, however, the currents were observed to be highly variable, and therefore it was not possible to assess their effect on the movements of the fish.

On the basis of releases which were made at the end of the Bay (release station No. 3 in Fig. 1), it would appear that the white bass move along the shore and find their spawning area through recognition of the local environment. The orientation behavior of the white bass near the spawning grounds needs more intensive study. Just how the north tendency, Type II homing, is combined with Type I homing, or if Type III exists, leaves room for further exciting discoveries.

The fish which were set free on cloudy days, and those to which plastic opaque eye-

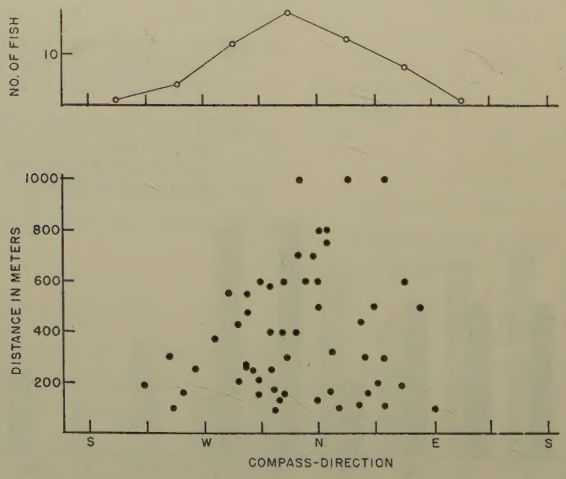

FIG. 3. Direction take-off and distance traveled, after one hour, by white bass from a mid-lake release point (Station 1, Fig. 1). Strength of current ignored.

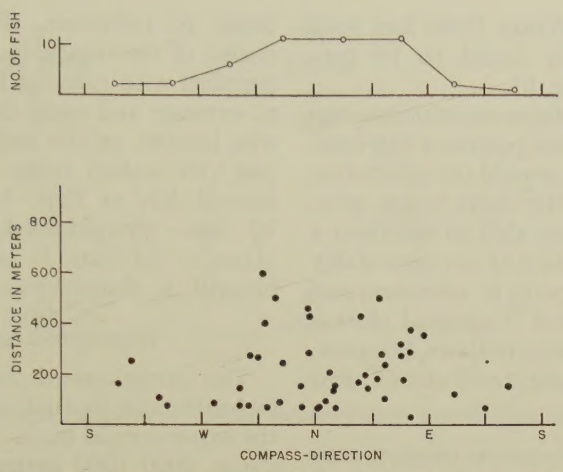

FIG. 4. Direction take-off and distance traveled, after one hour, by white bass from a mid-lake release point (Station 1, Fig. 1). Corrected for drift by currents.

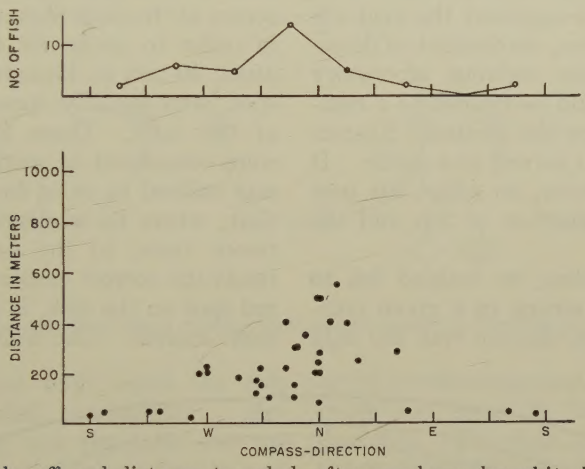

FIG. 5. Direction take-off and distance traveled, after one hour, by white bass released between Maple Bluff and Governor's Island (Station 2, Fig. 1). Current ignored.

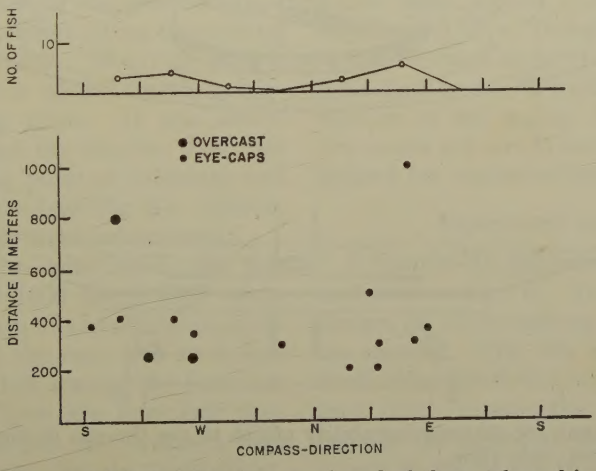

FIG. 6. Direction-tendency of blinded fish (eye caps) and of those released in mid-lake (Station 1, Fig. 1).

caps (See method of Wisby 1958) had been attached, were usually found to be randomly distributed (Fig. 6).

An appraisal of the above experiments suggests that the white bass possess a sun-compass mechanism which is used for orientation in open water where the shore is not seen. In other words they are able to maintain a constant compass direction at any time of day by using the sun as a point of reference, and therefore must possess a "biological clock." Upon reaching the vicinity of shore, however, they appear to locate their specific home areas by other methods.

EXPERIMENTS ON SUN-COMPASS ORIENTATION

To investigate the existence of a sun-compass mechanism, laboratory studies were necessary in order to augment the field observations. Therefore, we decided to determine if a fish, under rigorous laboratory experimentation, could be trained to a compass direction. Here the methods Kramer (1952) used for birds served as a guide. It was necessary, however, to adapt his procedures to the peculiarities of fish and the aquatic medium.

In the initial studies, we trained fish to either take food or escape in a given compass direction, where the sun was the only point of reference. Figure 7 shows the design of the rotatable test tank which was supplied with running Lake Mendota water at summer and early fall temperatures. It was located at the end of the Laboratory pier, 26 meters from shore. The experimental fish in Exp. A was an immature 70 mm pumpkinseed, *Lepomis gibbosus* (Linn.). In Exp. B an immature 70 mm bluegill, *L. macrochirus* Raf., was trained.

Experiment with Fish A

The pumpkinseed was maintained in a separate tank located out-of-doors and near the experimental tank. Every forenoon between 0900–1000 hours CST the fish was transferred to the training tank. At each trial it was placed in a small cage in the center of the tank from which it was released in order to make its choice. Four white disks, 40 mm in diameter and bearing a red spot, were equally spaced around the edge of the tank. These four compass points were considered as scoring zones. The fish was trained to swim from the center of the tank, where he was momentarily caged between tests, to the south-disk. When it made the correct choice and snapped at the red spot on the disk, it was rewarded with a meat morsel. The training process was re-

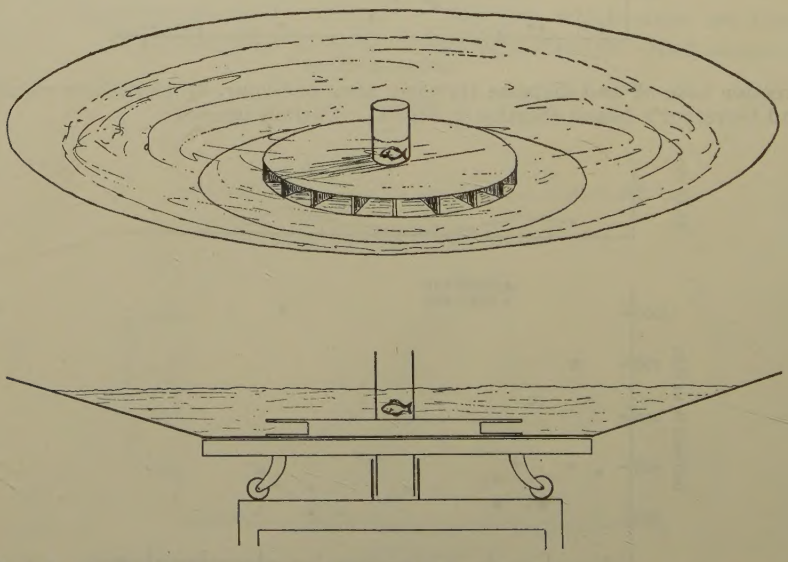

FIG. 7. Training tank for determining ability of fish to use the sun in direction location: *top*, as seen from above; *bottom*, side view.

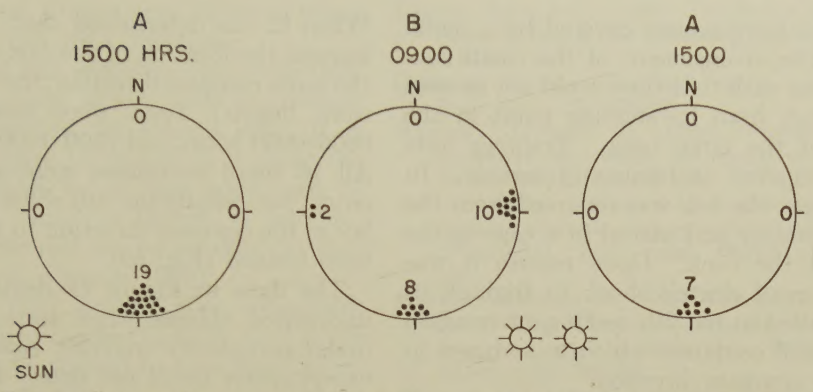

FIG. 8. Scores where cups casting shadows served as feeding (reward) sites.

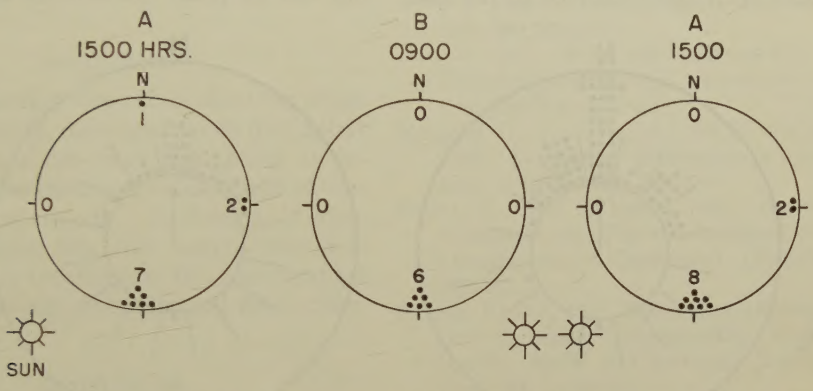

FIG. 9. Scores where discs casting no shadows served as feeding (reward) sites.

peated several times daily until the fish chose the correct disk consistently. Between each training test the tank was rotated in order to prevent orientation to any marks in the tank. The observer changed his position each time for the same reason.

An additional comment about the scoring zone might be pertinent. In earlier experiments, tea cups were used as feeding containers and scoring zones. It was determined, however, that the shadow they cast provided a confusing point of reference and caused erratic choices (see Fig. 8). Hence, the shadowless disks were substituted.

After training was established, the fish was tested at 1500–1600 hours CST when the azimuth of the sun was at about the same angle in relation to the east disk as it had been to the north disk during the forenoon training period. There are here two possible choices: (a) the fish may choose a disk at a constant angle to the sun (west disk), or (b) it will compensate for the sun's movement and swim in the trained compass-direction. The data (Fig. 9) suggest that this individual behaved as in (b), indicating that it possesses a sun-compass mechanism which also implies a type of orientation coordinated by a "biological clock."

At this stage in the study the out-of-doors tests had to be discontinued owing to the lateness of the season; hence the relatively few scores are not so convincing as would be desired for statistical reliability.

Experiment with Fish B

A completely different training technique was used on Fish B. In this type of test an escape, or cover-seeking response, was used for scoring. The fish in this arrangement lived throughout the experiment in the test tank (Fig. 7) under the open sky. The fish usually attempted to seek cover and found it only in one of the 16 small compartments,

the others having been covered by a metal band. The arrangement of the small containers was such that they could not be seen by the fish from its starting point in the middle of the large tank. Training tests were conducted at frequent intervals. In this process the fish was removed from the small container and placed in a cage in the center of the tank. Upon release it was given a small electric shock to frighten it. This resulted in the fish seeking cover again in the small container which was always in the same compass direction.

Trials were then conducted with all 16 of the boxes open and available to the fish. When it was determined that the fish had learned the location of the box which lay in the same compass direction, the critical tests were begun. Tests were made between 0800–0900 hours and 1500–1600 hours CST. All 16 small containers were available for entry, but usually the fish chose those which lay in the compass direction to which it had been trained (Fig. 10).

The data in Figure 11 demonstrate the unoriented choices when tests were made under completely overcast skies when the experimenter could not detect the presence of the sun. This clearly proves that the sun was its point of reference and that the

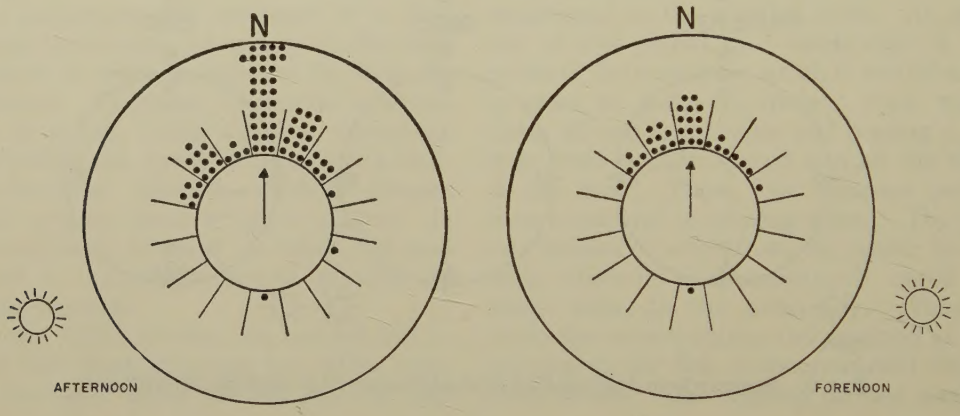

FIG. 10. Scores of Fish B trained to north; *left*, tested in the afternoon with 16 possible choices; *right*, tested in the forenoon with 16 possible choices.

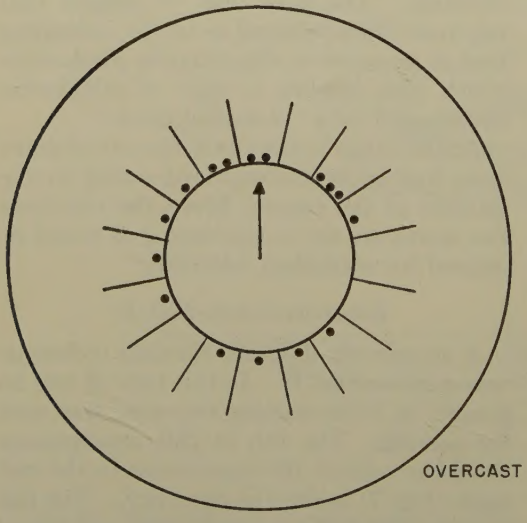

FIG. 11. Scores of Fish B tested under completely overcast sky on two different days.

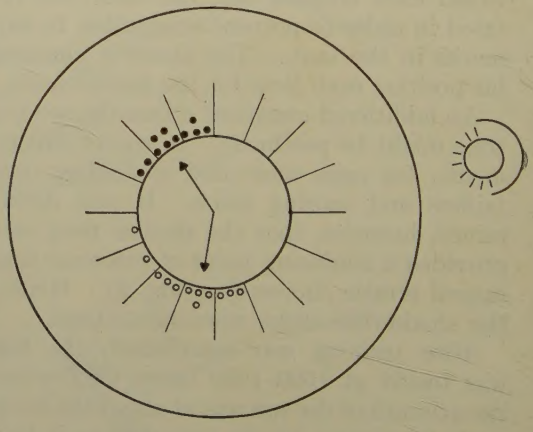

FIG. 12. Scores of Fish B using an artificial light, where the altitude was the same as the sun.

fish had learned to escape at different times of the day in the same direction.

The crucial and definitive test was then conducted, namely, to substitute an artificial "sun" in-doors for the natural sun. It is clear from Figure 12 that Fish B chose the hiding-box which lay in that same angle to the artificial "sun" as though it were responding to the real sun out-of-doors at that time of day (cf. Kramer 1950).

Since the 1957 studies were completed, initial investigations have shown that white bass can also be trained to a compass direction. These results will be described in detail in a future publication (Braemer and Hasler 1958).

CONCLUSION

Type II homing was established for white bass. Moreover, our experiments prove that three species of fish have the ability to locate a compass direction at any time of day with the aid of the sun. In doing so it is implied that some fish, like many other animals, have a mechanism for sun-compass orientation which is coordinated with a biological clock.

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Orientation of fish to gravity[1]

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ABSTRACT

The equilibrium position about the transverse axis was studied in three species of Characinoidei, following the original work of von Holst.

The equilibrium position was measured in intact fish and in fish with one utricular statolith removed, under conditions in which the weight was increased from 1.0 to 2.2 g through centrifuging. Some specimens were fixed and cleared so that the position of the utricular statoliths relative to the long axis could be photographed.

The experiments show: (1) The equilibrium function is a feedback process of peripheral and central factors, with the sensory receptor built into the system as the external control element. (2) Removal of one utricular statolith results in a marked weakening of the peripheral equilibrium component, but is not equal to an exact halving as should be expected from the results of other workers. This suggests that one of the other statoliths may be acting in maintaining the equilibrium position around the transverse axis. (3) The direct measurement of the morphological angle of the utricular statoliths imbedded in the body of the fish was compared with the calculated value obtained by centrifuging the whole animal.

A. *Positional reactions*

When we turn a living fish around its longitudinal or transverse axis thereby forcing it to assume an abnormal angle, we observe changes of position of the eyes and compensating movements of the fins, producing a torsional moment adequate to bring the animal back into its normal position (Figs. 1a and 1b). The receptors for controlling such postural responses are the utricular statoliths in the labyrinth (Fig. 2). The location of these receptors has been demonstrated in many experiments in which the various parts of the statolith system have been eliminated one by one (see Lowenstein 1957).

The statolith of the utriculus is a flat stone of high specific gravity. It lies on the hairs of a sensory epithelium which is covered with an elastic gelatinous material. For a long time it was not known just how the statolith of the utriculus functioned in enabling the animal to maintain its vertical position. It was thought that it either stimulated the epithelium by exerting pressure on it, or that the statolith might stimulate the sensory epithelium by a force parallel to its surface (shearing force) when the body was turned. Working with free swimming fish, von Holst (1950) succeeded in demonstrating what the effective stimulus was. In his studies he used fish in which the statolith of the utriculus lies in a horizontal plane when the fish is in its normal upright position. He had earlier discovered that some species of bony fish also use their eyes for orientation in space (v. Holst 1935). When illuminated from one side, they swim with a longitudinal tilt toward the light (Fig. 1c) without showing the torsional moments described. If the statoliths of both utriculi are removed and the fish are subjected to diffuse light from all sides, or in the absence of light, such fish are completely lacking in orientation, turning around aimlessly in all directions. If, however, they are illuminated from one side, they will swim with their backs directed exactly towards the light. In horizontal light beams, for example, their dorsoventral axis will be horizontal, *i. e.*, the fish is on its side. If an intact fish is illuminated laterally, its dorsoventral axis will be oblique, the angle assumed being the resultant between the torsional moment produced through the

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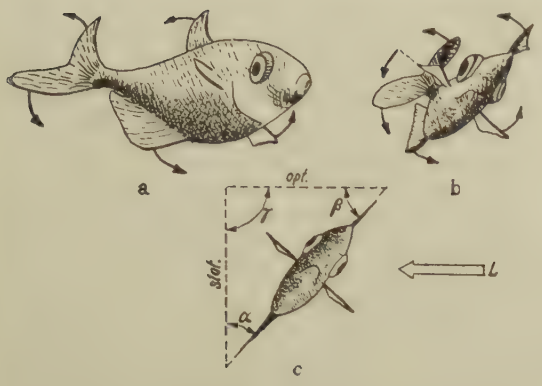

FIG. 1. Equilibrium reactions of a fish tilted around its longitudinal axis to the left side. *a*. Side view. *b*. Front view. The arrows show the direction of operation of the fins. *c*. In horizontal light beams (L), the reactions seen in *a* and *b* disappear. The assumed tilt toward the light is the equilibrium position. α = angle between dorsoventral axis and vertical; β = angle between dorsoventral axis and direction of light; γ = angle between direction of light and vertical (after von Holst 1950).

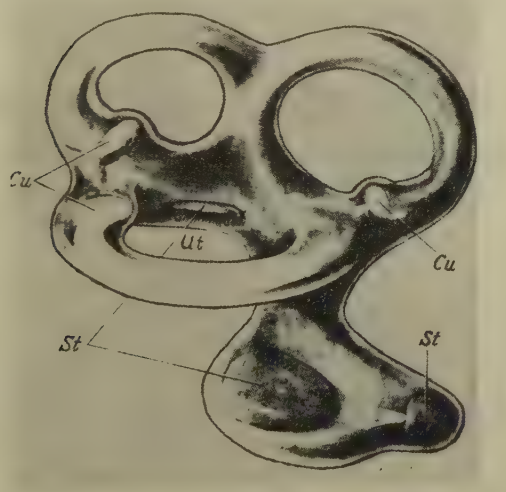

FIG. 2. Drawing of fish labyrinth: *St*-statoliths, *Cu*-cupulae of the semicircular canals. The statolith lying horizontal on its epithelim is the utricular statolith (*Ut*) (after von Holst 1950).

statoliths and the tendency to turn its back to the light. Thus it seems clear, in the described reaction, that the eyes, responding to the direction of illumination, and the utricular statoliths are the only sense organs involved in providing information to the central nervous system for bringing about positional adjustments. It also seems clear that in the absence of one of these sources of sensory input, the behavior of the fish is controlled by the other.

B. *The adequate stimulus of the utricular statoliths*

From the introductory observation (Figs. 1a and 1b) on a fish which is not in equilibrium we might expect that, if illuminated from one side, it would show less tilting response when the "weight" of its statoliths was increased. This means that the angle between its dorsoventral axis and the vertical decreases. It is possible to increase the weight acting upon the fish at any quantitative rate by the addition of a centrifugal force. In such an experiment the fish swims within an experimental tank, against a stream of water, so that it remains in one spot. The tank is mounted on an arm of a centrifuge so that it can rotate freely when the centrifuge is operated. The fish assumes a position such that its longitudinal axis is in a plane vertical to the resultant of the force of gravity and the

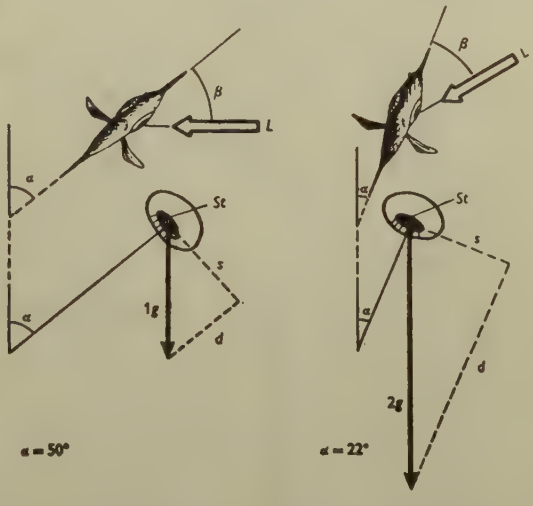

FIG. 3. Sketch to illustrate the behavior of the fish with g doubled when the light intensity and the angle of light entry into the eye is kept constant. The measured angle of inclination is so changed that the shearing force (*s*) acting perpendicular to the sensory hairs remains constant. The pressure component (d) may increase considerably. 1g is the normal gravitational field (after von Holst 1950).

centrifugal force, while the dorsoventral axis of the fish is parallel to the resultant. This resultant force will be measured in g units, 1 g being equal to the normal gravitational field. Laterally to the fish a luminescent sidewall is mounted, so that the dorsoventral axis of the fish deviates at a certain angle from the direction of g.

The experimental fish chosen were angel fish (*Pterophyllum eimeckei*) and black tetras (*Gymnocorymbus ternetzii*) which are laterally compressed, and when observed from their anterior or posterior end it is possible to measure very accurately the angle which the fish makes to g (von Holst 1950).

It can be assumed that in each case for fish in balance the angle measured represents a condition of equilibrium in the central nervous system between the information coming from the eyes and the information coming from the utricular statoliths. Therefore, torque resulting from stimulation of the eye is equal to that resulting from stimulation of the statoliths, but of opposite sign. If in such experiments the angle of light entry into the fish eye and the light intensity are kept constant (Fig. 3) while g is increased, then one finds that g times sine α = constant (the sine rule referred to below). The stimulus coming from the sensory epithelium of the utriculi must remain unchanged in the measured position of the fish, for otherwise the fish would be off balance. The component equal to g times sine α, which is the effective shear component, remains constant with an increase in g, although the pressure component increases. Maintaining a constant optical situation while increasing the weight of the statoliths causes the tilted fish to orient in such a way that the shearing portion remains constant. Thus it is the shear component and not the pressure component which is the physiologically important sensory input.

Let us put this sine rule to use in the normal field of gravity (g). In the upright position of a "normal" fish the shearing force component is zero (Fig. 4). If the fish tips to one side the stimulus to restore

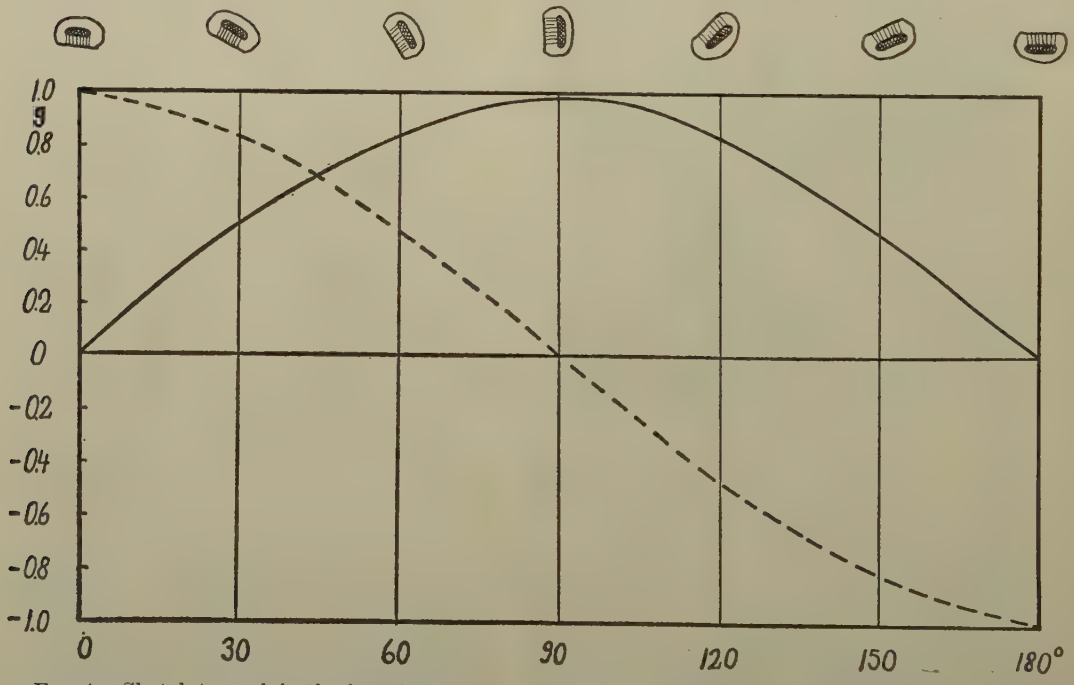

FIG. 4. Sketch to explain the function of the sensory input, depending on the position of the utricular statoliths. α = angle between dorsoventral axis and vertical. The solid line represents the effective shearing component (sine function); the dotted line represents the ineffective pressure component while the fish is turned around its long axis in the normal field of gravity (1g) (after von Holst 1950).

the normal position increases with the sine of the angle of inclination. Accordingly the stimulus reaches its maximum value with a 90° inclination to the vertical. As the angle is further increased, the stimulus is decreased with the sine of the angle (Braemer 1957). As a result of this relationship the stimulus increases at a maximal rate with a small deviation from the normal vertical position. Thus the fish is able to regulate its position in a very precise way, when near its normal posture.

In experiments conducted by Lowenstein and Roberts (1950), unit responses of the utriculus macula were obtained at intervals throughout a complete revolution about the longitudinal axis in the elasmobranch *Raja clavata*. The discharge frequency shows a maximum near a position in which the labyrinth under observation lies uppermost, but a minimum near the spatially opposite position. Besides units which have static discharge rates strictly corresponding to certain spatial positions of the labyrinth (position receptors), there are other end organs in the macula which react by a change in their discharge rate to the movement as such, irrespective to the direction of change, returning to a basic discharge rate whenever the head has come to rest in a new position (out-of-position receptors).

C. *Fish with a non-horizontal position*

We have raised the question as to how those fish in which the normal position deviates from the horizontal, by rotation about the transverse axis, use these sensory organs. There are two possibilities (Fig. 5): (1) the utricular statoliths may lie in a horizontal plane when these fish are in their characteristic position, or (2) the plane of these statoliths may not be horizontal in the normal position of the fish. In the first case artificially raising the gravitational force will only increase the ineffective pressure component, while the shearing on the sensory hairs will not be increased. In other words this procedure would not re-

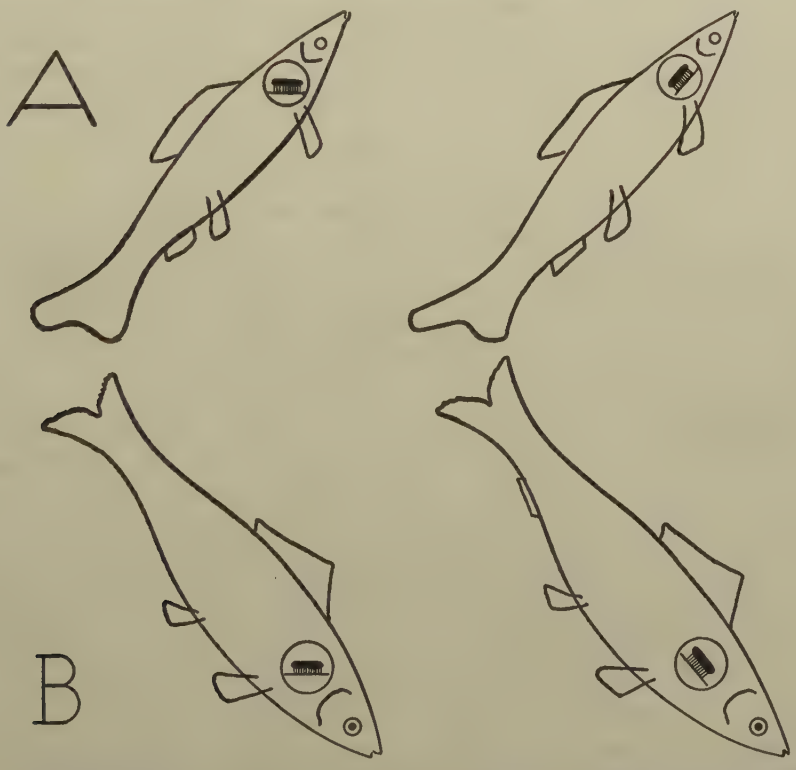

FIG. 5. Possible position of utricular statoliths in fishes with slanted normal position. *A*. tail-stander. *B*. headstander.

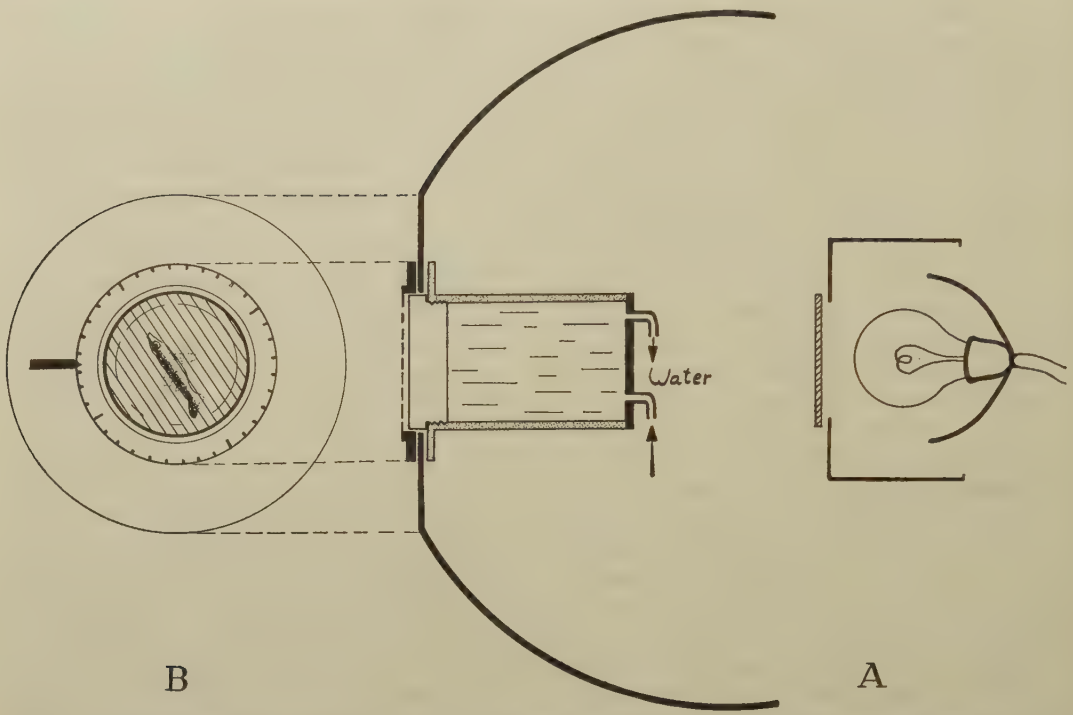

FIG. 6. Experimental apparatus. *A*. longitudinal section. The plexiglas container for the free-swimming fish has roughened walls and is surrounded by a white screen to make the light diffuse from all sides. *B*. cross section. If the scaled ring is turned so that the threads are parallel to the fish the hand points on the angle of inclination.

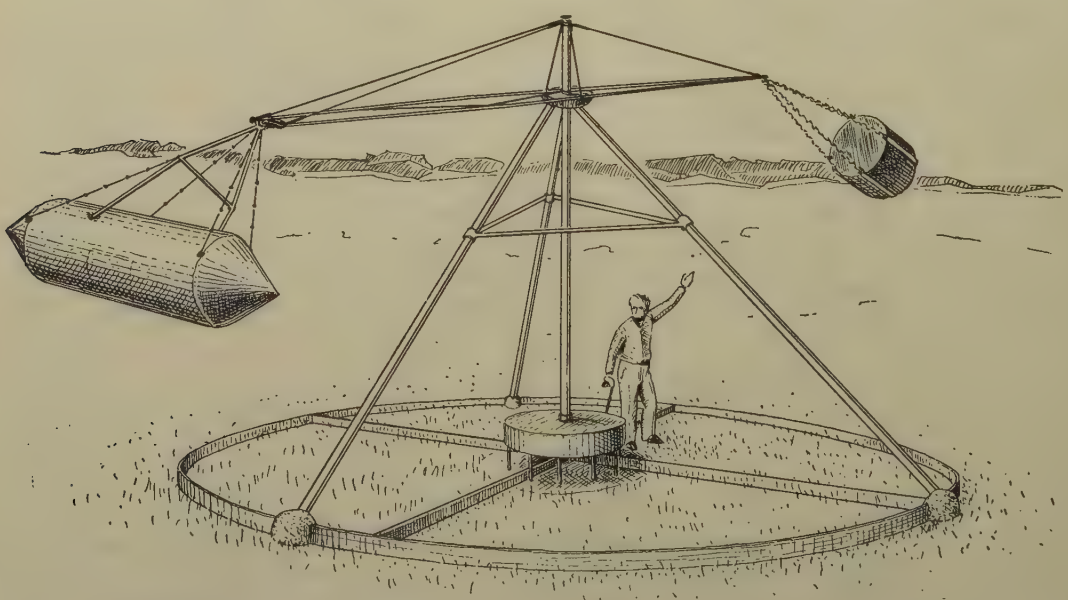

FIG. 7. Drawing of the centrifuge in operation. In the left gondolar shaped container is the observer with the experimental apparatus. The right container serves as counterweight.

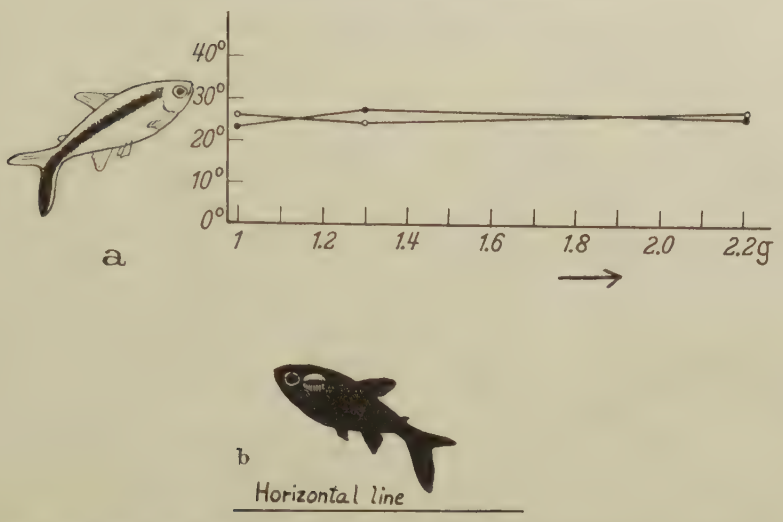

FIG. 8. *a*. The angle of inclination of *Thayeria Boehlkei* Weitzmann against the "horizontal" is independent of g. The three points in each curve are average values of at least 10 single recordings for a single fish. *b*. Horizontal position of the utricular statolith in the head of *Thayeria* from measurements in fishes made transparent.

sult in any change in the normal deviation of the fish about the transverse axis. The information coming from its statoliths would be always the same. In the second case, however, the shearing force of the statoliths can be increased by adding to the gravitational force, resulting in a measurable decrease of the angle which the body axis makes with the horizontal.[3]

In experiments designed to test these possibilities, fish were placed in a chamber illuminated from all sides by diffuse light (Fig. 6). With the fish in this chamber the angle of deviation from the direction of g could be measured. This simple apparatus, along with the observer, was placed in a centrifuge (Fig. 7) in which forces up to 2.2 g could be obtained.

After these experiments some fishes were killed and treated to make them transparent (Ahrens 1950). The position of the statoliths in the fish was determined in the following way: the transparent fishes were photographed under the binocular microscope and the angle which the statolith of the utriculus made with the longitudinal axis of the fish was measured.

In systematic series the species under study were photographed with a flash bulb in complete darkness with a robot camera, in order to find out the swimming angle of the fishes under these conditions.

D. *Quantitative experiments on three species*[4]

1. *Thayeria Boehlkei* Weitzmann (formerly *Th. obliqua* Eigenmann) (penguin fish) normally swims at an angle of 25° off horizontal during both day and night. When g is increased, this angle does not change. This means that that aspect of the weight of the utricular statoliths which is physiologically important (shearing force) cannot be increased by centrifuging. The theory predicts that the utricular statoliths lie horizontally on the sensory epithelium while the fish is in its normal posture. This conclusion was verified by direct observation of the utricular statoliths in cleared preparations (Figs. 8a and 8b).

2. *Poecilobrycon* (*Nannobrycon*) *eques* Steind (pencil fish) swims on more of a

[3] In the experiments with the centrifuge, the "horizontal" is perpendicular to g. The centrifuge was designed by Dr. E. von Holst.

[4] The taxonomy of the species is not clear. We followed the helpful advice of Dr. Werner Ladiges, Hamburg, Germany.

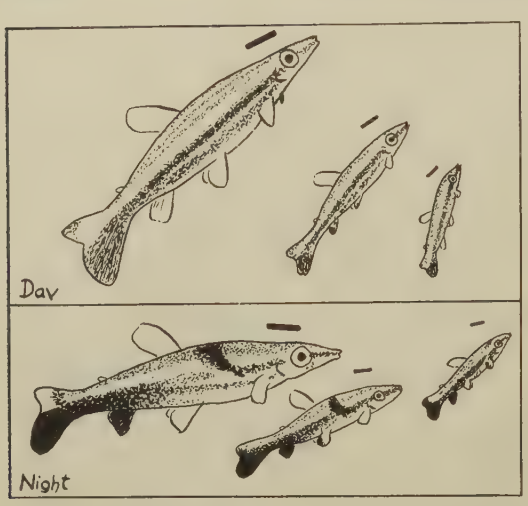

FIG. 9. Position and coloration of different sized specimens of *Poecilobrycon* (*Nannobrycon*) *eques* during day and night. The lines over the individual animals indicate the position of the utricular statoliths. At night the fish swim close to the surface with the statoliths approximately horizontal.

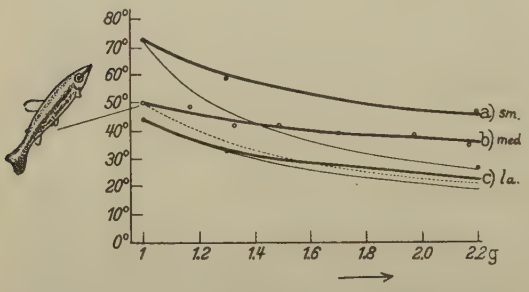

FIG. 10. Angle of inclination of *Poecilobrycon* (*Nannobrycon*) *eques* against the "horizontal" as a function of g in different sized specimens (small *sm.*, medium *med.*, large *la.*). Data are average values of many individual recordings with ten animals in each set. The thin curves indicate how the fish would behave if the utricular statoliths were arranged parallel to the long axis of the fish. The thick curves fit the actual statolith inclination best.

slant during the day than at night (Fig. 9). This was demonstrated to be due to the presence of light during the day. Three age groups were studied since younger stages swim at more of a slant than older ones. When centrifuged in uniform diffuse light, the angle which the fish makes to the horizontal is reduced (Fig. 10). Therefore the statoliths of the utriculi cannot be horizontal while these fish are in their normal daytime posture.

It should be remembered that the optical conditions remained constant in the above experiments. The fish were exposed to diffuse light from all directions. Immediately following the measurements made with the increased weight, the fish again swam at the same angle as before the experiment. We assume therefore that the condition of the central nervous system is not changed. Therefore, since it has also been shown that the adequate stimulus is the shearing force

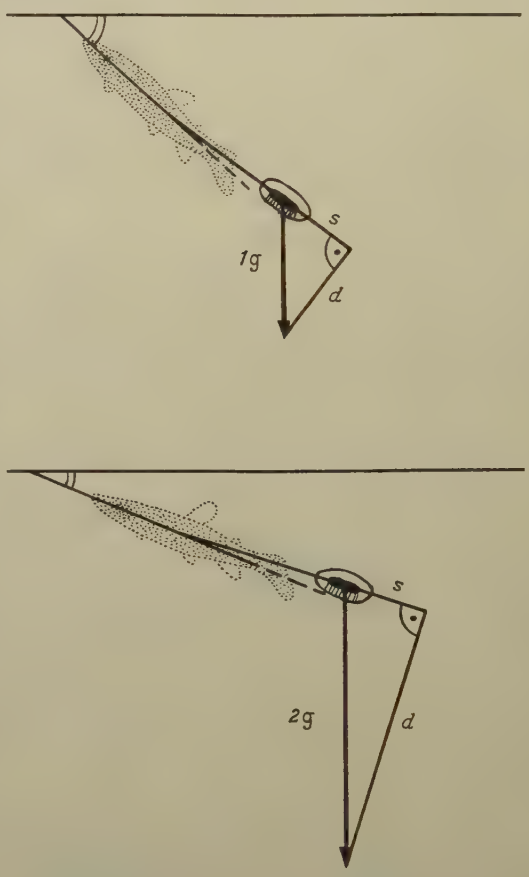

FIG. 11. Sketch to explain the behavior when g is doubled. The dashed line is the continuation of the long axis of the fish. The angle between the dashed line and the solid line is the calculated angle of the utricular statolith in the fish. The angle of inclination of the utricular statolith is so changed that the shearing force (s) acting vertically to the sensory hairs remains constant. The pressure component (d) may increase considerably.

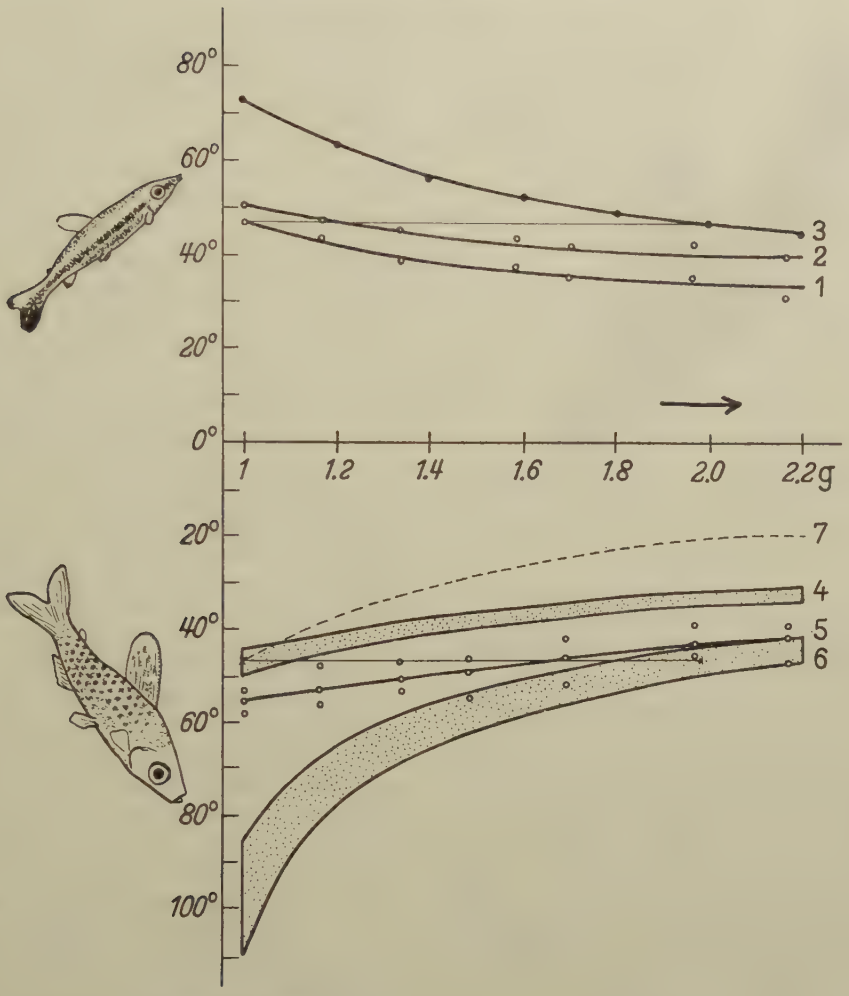

FIG. 12. Angle of inclination against the "horizontal" as a function of g. 1) Average curve from five intact specimens of *Poecilobrycon*. 2) Same fish as in 1), one statolith removed. 3) Expected behavior of animals in 1), after removing one statolith with linear stimulus evaluation and addition of the torque caused by both statoliths (theoretical curve). 4) Five intact *Chilodus*. The scattered field includes the extreme measured values. 5) Average curve for one *Chilodus* after removal of one utricular statolith. The individual points give the extreme values. 6) Expected behavior of animals in 4) after removing one utricular statolith with linear stimulus evaluation and addition of the torque caused by both statoliths (theoretical field). 7) Curve indicates how intact *Chilodus* would behave if the utricular statoliths were arranged parallel to the long axis of the fish.

(von Holst 1950), it is easy to calculate just how the statolith of the utriculus must be positioned in the fish (Fig. 11). Direct measurements with cleared specimen confirmed this conclusion. On this basis it was also determined that the angle of the utricular statoliths to the long axis of the fish must be reduced in the course of its life. We have already stated that these fish swim at less of a slant during the night than in the daytime. We have taken photographs of a large number of fish during the night. In this way we determined that the position of the fish at night is such that their utricular statoliths lie approximately horizontally (Fig. 9).

3. *Chilodus punctatus* (headstander) is the third species we have investigated. Only five of these animals were available. They swim with their heads downward at an angle

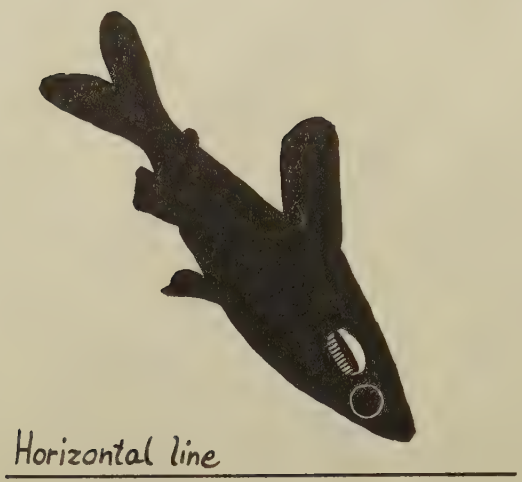

FIG. 13. Position of the utricular statolith in *Chilodus punctatus* based on one transparent fish.

of 45–50° during both day and night. In the centrifuge this angle is reduced (Fig. 12, Curve 4). We assumed therefore, as in the case of the pencil fish, that in this animal the statoliths of the utriculi must lie at a slight angle from the long axis of the fish, but in the opposite sense to the one found in *Poecilobrycon* and *Thayeria*. The cleared preparation of only one fish, however, did not confirm this conclusion. In this case the morphological angle of the statoliths deviated by 20° from the calculated value (Fig. 13). This discrepancy is discussed later.

E. *Relations between central nervous system and receptor*

We should like now specifically to consider the relation between the equilibrium receptors and the central nervous system. This relation is especially clear in the behavior of *Chilodus* and *Poecilobrycon*. As we have seen, the normal position is not necessarily the position in which the statoliths lie horizontally. From simple observation we know that all animals, including man, are able to maintain positions which deviate from the normal equilibrium position. For example fish can assume a vertical position, and man also changes his normal position when swimming under water. We are accustomed to say in these cases that the "righting reflexes" have been inhibited, but this is not the case. For in these positions there is a constant orientation to gravity which is lacking if the organs of equilibrium are destroyed. These positions are maintained by means of the same "reflexes" which operate in keeping the animal in its normal position relative to gravity. This means, however, that there is not simply a righting reflex controlled by the organs of equilibrium alone, but that the equilibrium function is a "feedback" process of peripheral and central factors, with the sensory receptor built into the system as the external control element.

Impulses are sent from the equilibrium center to the effectors, which, in turn, cause the animal to rotate. This produces an excitation in the static organs. If the impulses from the central nervous system and the sensations coming from the statoliths are equal in intensity but opposite in sign, the animal will be in equilibrium. This mechanism enables the animal to keep each position, dictated by the central nervous system through the same "reflexes" even against mechanical disturbances (von Holst and Mittelstaedt 1950, Schoene 1957, Braemer and Braemer 1958). This will also be demonstrated in the next example.

Von Holst determined the manner in which the left and right statolith of the utriculus work together. A fish with one utricular statolith removed tilts much more strongly toward a horizontal light beam than before the operation. Von Holst obtained an equal mechanical balance component whether he applied a certain shearing force to both statoliths, or doubled the shearing component after removal of one statolith. The obvious conclusion is that the impulses coming from the left statolith of the utriculus are simply added to the impulses coming from the right side.

F. *Experiments after removal of one utricular statolith*

We have also studied the interrelationship of the right and left statolith in orientation of our fishes about the transverse axis. According to the studies of von Holst, removing one of them should reduce the

static equilibrium component to one-half its normal value. Therefore, we expected that following the destruction of half the sensory cells half the afferent impulses would be removed. A fish lacking one utricular statolith will attempt to achieve an equilibrium position with the remaining statolith according to the consideration given above. In this condition the last two species discussed should show considerably larger angles of inclination to the horizontal, since they should attempt to double the shearing force of the single remaining utricular statolith. Consequently by applying 2 g we should obtain the same angle as that produced by 1 g before the operation.

We found in both cases a marked weakening of the static equilibrium component (Fig. 12, Curves 2 and 6), but these results are not in quantitative agreement with the expected result. This is the present stage of our investigations.

One particular feature of *Poecilobrycon* and *Chilodus* as compared with other species is that their normal position does not conform to the zero position of their statoliths. So far as is known in other species the statolith is imbedded in the body so that it lies horizontal or nearly so in the normal position, as for example in *Thayeria*. Upon deviation from this zero position the excitation rises sharply (sine rule Fig. 4). This causes a high accuracy in balancing when the fish is near its normal position. In *Poecilobrycon* and *Chilodus*, however, the normal position deviates from this zero position of the statoliths. As a consequence the stimulus will not be as strong when deviations occur. We think that there might exist a relation between the behavior of animals with one statolith removed and this phenomenon. In *Chilodus* the morphological angle of the statoliths deviated also from the calculated value. We assume that one of the other statoliths may be acting in maintaining the equilibrium position of these two species. The advantages of cooperation between the utricular statoliths and those in the lagena or in the sacculus are evident. The precision of adjustment would be improved. Our studies will continue in this direction.

SUMMARY

The equilibrium-position about the transverse axis was studied in three species of Characinoidei, following the original work of von Holst.

A. *Thayeria Boehlkei* Weitzmann (penguin fish) normally swims 25° off horizontal, during both day and night. Increasing its weight does not change this angle. This means that the shearing component of the utricular statoliths, which is physiologically effective, is not increased by centrifuging. This, in turn, is only possible if the statoliths lie horizontally on the sensory epithelium while the fish is in its normal posture. This conclusion was verified by direct observation of the utricular statoliths in cleared preparations.

B. *Poecilobrycon* (*Nannobrycon*) *eques* Steind (pencil fish) swims on more of a slant during the day than at night. This proved to be due to the presence of light during the day. Three age groups of the animals were studied since younger animals swim at more of a slant than older ones.

When centrifuged in uniform diffuse light, the angle the fish takes to the horizontal is reduced. This indicates that during the day the utricular statoliths lie at a definite angle to the horizontal. This angle decreases as the fish becomes older. Direct observations of cleared preparations of the three age groups confirm this conclusion.

At night *Poecilobrycon* lies at such an angle that the utricular statoliths are approximately horizontal.

Removing one utricular statolith does not result in reducing the equilibrium response by exactly one half, as would be expected on the basis of earlier studies.

C. *Chilodus punctatus* (headstander) directs its head downward at an angle of 50° during both day and night.

In the centrifuge this angle is reduced. It was calculated that in this animal the utricular statoliths must lie at a slight angle from the long axis of the fish, but in the opposite sense from the angle found in *Poecilobrycon* and *Thayeria*. The cleared preparation did not confirm this conclusion.

Removing the utricular statoliths on one side resulted in a marked weakening of the

peripheral equilibrium component. But again in this fish the equilibrium response was not reduced by exactly one half.

The functions of the gravitational sense organs in these fishes and their connections with the central nervous system, as described, are supported by the results of other experiments. The incomplete halving of the peripheral equilibrium component, following the removal of one utricular statolith, suggests that other statoliths are involved, as does the fact that in two species (*Poecilobrycon* and *Chilodus*) they do not lie horizontally in the normal position.

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The Salton Sea. Physical and Chemical Characteristics[1]

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ABSTRACT

The Salton Sea of California lying below sea level in a desert basin, serves as a sump for irrigation water diverted from the Colorado River. The present Sea reached its maximum size and highest surface elevation at the time of its formation in 1907. Due to evaporation at the rate of six feet per year the surface elevation fell from −195 feet to its minimum of −240 feet in 1948, the surface area decreased from about 500 square miles to about 320, and the salt content (salinity) increased from 3.6‰ to about 40‰. Since 1948 increased "wastage" of water into the Sea has raised the surface to −235 feet (1956) and diluted the waters to a salinity of about 33‰. Although the total salinity is approximately the same as that of the oceans the waters of the Sea contain a different proportion of ions, and the pH is slightly higher.

The annual range of water temperature is from 10° to 36°C. In spring the surface may become as much as 5° warmer than the bottom, but since the maximum depth of the Sea is about 12 meters, mixing by winds rapidly removes differences between surface and bottom; during most of the year (August to May) there is less than 1°C difference between surface and bottom. Concentrations of dissolved oxygen ranged up to 11.8 mg/L at the surface and 9.3 mg/L at the bottom during winter. During calm periods in summer, however, the water at depths below 8–9 meters became anoxic for periods as long as 1–3 days; the surface waters, too, became depleted of oxygen, but for only an hour or so at about dawn. Concentrations of sulfide as high as 85 μg-at./L, and of ammonia as high as 50 μg-at./L were found during the anoxic periods in summer.

INTRODUCTION

The present Salton Sea covers an area of about 340 square miles in southern Riverside County and northern Imperial County, California. (The center is about 33° 20′ N and 115° 50′ W. See Fig. 1.) The Sea lies in the Salton Basin, which is a depression with a maximum depth of 273 feet (83 meters) below mean sea level, north of the delta of the Colorado River. The delta separates the basin from the Gulf of California. According to Buwalda and Stanton (1930) the trough sank from above to below sea level during or since the formation of the delta. The Sea is therefore not a cut off arm of the ocean. The salts present in the Salton Sea are the result of evaporation of Colorado River water.

An old shore line, at an elevation approximately 30 feet above mean sea level (at which height the lake would spill into the Gulf), records a previous lake which covered 2200 square miles and extended from the Mexican border to the Indio Hills. This previous lake, which is judged to have been of fresh water from such evidence as the species of mollusk shells present, was called Lake Cahuilla according to Indian legends. It existed during a "pluvial" period in the late Pleistocene according to Cockerell (1946), and is referred to as Lake LeConte by Hubbs and Miller (1948) whose paper includes an excellent list of references to geological studies of the region. Since the days of Lake Cahuilla there have been numerous but less extensive lakes formed by

[1] With the objective of establishing a sport fishery in the Salton Sea, the Wildlife Conservation Board, State of California Department of Fish and Game, contracted with Fisheries, Department of Zoology, University of California, Los Angeles, to conduct a survey of the Sea. A complete report will be published elsewhere; this report summarizes some of the physical and chemical findings. The help of Dr. Boyd W. Walker, who supervised the project, is gratefully acknowledged, as is the assistance of an Advisory Committee of the Department of Fish and Game, which consisted of Frances Clarke, Phil Douglas, John Fitch, Scott Soule, and John Radovich. Acknowledgments are also due the following for making data available: H. F. Blaney, A. J. Boles, M. R. Huberty, J. S. Reger, T. A. Sprink, H. C. Troxell, Lowell Weeks, and L. V. Wilcox.

inflows from the Colorado River whenever it shifted its course through the delta and drained into the basin rather than into the Gulf. One such filling was recorded in 1891. Then, as on previous similar occasions, the limited amount of water soon evaporated leaving a desert basin playa with a sufficient deposit of salt to allow salt mining to be carried on prior to 1905.

The present Salton Sea dates from the spring of 1905 when the main flow of the Colorado River was accidentally diverted into the Salton Basin by way of irrigation canals in the newly reclaimed Imperial Valley. The break was not repaired until February of 1907. During the two-year period the basin was filled to the −195 foot level, creating a lake with a maximum depth of 79 feet (24 meters) and covering 515 square miles.

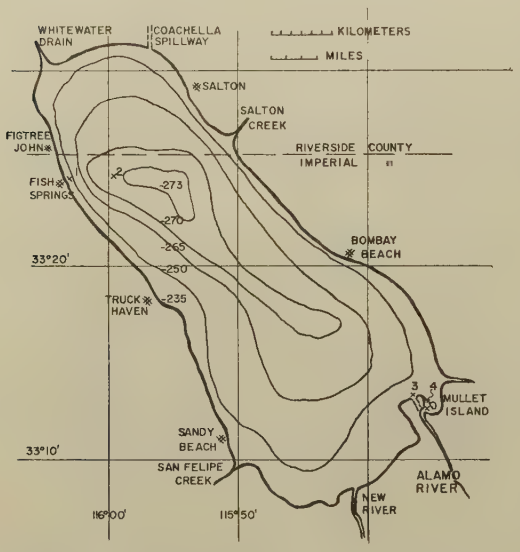

FIG. 1. Sketch of Salton Sea. Contours above −250 feet from U. S. Geological Survey, 1925. Contours below −250 approximate only, based on soundings by R. Arnal and U. C. L. A. Salton Sea Laboratory, 1954–1956. The four numbers show the location of stations mentioned in the section on chlorinity.

IONIC COMPOSITION

Records from the period of the formation of the Sea (MacDougal 1914) reveal that in 1907 the salinity was about 3.6‰, which was approximately five times greater than the salt content of the Colorado River which was 0.7‰ at Yuma, the inlet to the irrigation canal. The difference in salinity is believed due to the salts leached from the previous bottom deposit.

The ionic composition of the Sea has changed somewhat since 1907. Table 1 shows that the proportion of Ca has decreased slightly from the value in the original Sea, and greatly relative to the proportion present in the River. The proportions of

TABLE 1. *Ionic composition (‰) of Salton Sea water 1907 to 1955 and comparison with river waters, ocean, and Caspian Sea*

Date	Ca	Mg	Na	K	CO_3	HCO_3	SO_4	Cl	Analyzed	Total
1907[1]	0.099	0.064	1.110	0.023	0.066	—	0.476	1.697	3.55	3.65
1910[1]	0.137	0.098	1.893	0.035	0.064	—	0.764	2.809	5.6	6.04
1913[2]	0.197	0.161	3.231	0.035	0.113	—	1.246	4.739	9.72	10.025
1916[2]	0.298	0.272	5.289	0.057	0.114	—	2.079	7.876	15.98	16.47
1929[3]	0.196	0.588	3.921	as Na	0.102	—	1.37	6.39	12.47	14.43
1945[4]	0.850	0.920	11.345	—	0.207	—	6.924	16.241	36.48	37.37
1948[5]	0.804	0.992	11.824	0.192	0.021	0.171	7.55	16.99	38.55	40.425
1955[5]	0.764	0.951	9.938	0.224	0.021	0.159	6.806	14.422	33.29	33.68
Colo. R. (1907)[1]	0.072	0.022	0.138	0.015	0.091	—	0.20	0.138	0.68	0.696
Alamo R. (1951)[6]	0.157	0.113	0.410	0.011	—	0.199	0.529	0.66	2.08	2.256
New R. (1951)[6]	0.141	0.066	0.435	0.01	—	0.209	0.444	0.605	1.91	1.93
Ocean[7]	0.413	1.27	10.55	0.38	—	0.014	2.649	19.15*	34.22	34.48
Caspian Sea[1]	0.29	0.77	3.17	0.07	0.047	—	3.10	5.46	12.9	12.94

Sources of data:

[1] Ross 1914
[2] Sykes 1937
[3] Coleman 1929
[4] Imperial Irrigation District Lab.
[5] USDA Salinity Lab., Riverside, Cal.
[6] California Division Water Resources Survey 1953
[7] Table 35, Sverdrup *et al.* 1942
* Chloride plus bromide

Mg and of SO_4, on the other hand, have increased gradually during the years since 1907. Na and K are now present in about the same proportions as in 1907; Cl has decreased slightly in proportion to total, and HCO_3 decreased from 13% of the total salts in river water to 1.9% in the original Sea and since then to the current value of about 0.5%.

At present the salinity of the Salton Sea (about 33‰) is only slightly below that of the oceans, but the waters contain relatively less Mg, K, and Cl; about the same proportion of Na; and more SO_4, Ca, HCO_3, and CO_3 than are present in ocean water. Although the present salinity of the Salton Sea is about three times greater than that of the Caspian Sea, the ionic composition of the two are fairly comparable. (Table 1). Apparently, evaporation of Volga River and Colorado River waters resulted in similar proportions of Ca, K, SO_4, and Cl, but with a greater proportion of Mg and less of Na in the Caspian.

With the exception of the aberrant analysis for 1929, the data of Table 1 indicate that the total salt content of the present Salton Sea increased gradually from 1907 to a high in 1948 when the salinity was 14 times greater than the original value. The increased salt content resulted from excess of evaporation over inflow. (The annual rate of evaporation is six feet or about 1.8 meters according to Blaney 1954.)

SURFACE ELEVATION

There have been fluctuations in the surface elevation of the Sea, due to variations in the amount of water inflow, as well as to the high rate of evaporation. Although the drainage area of the Salton Basin is quite extensive—about 7500 square miles—it is a desert region, so that the Whitewater, Salton, and San Felipe drainages are minor sources compared to the amount of water that enters the basin from the Colorado River. Inflow from the Colorado has depended on irrigation practice. When the diversion of the full flow of the Colorado into the Salton Sea ended in 1907, relatively little land was under cultivation in the Imperial and Coachella Valleys, and little water entered the Sea. Records of the U. S. Department of the Interior, Geological Survey, show that the surface fell from −195 feet in 1907 to −250 feet in 1925. The level fluctuated between −250 and −243 feet during the next ten years. After com

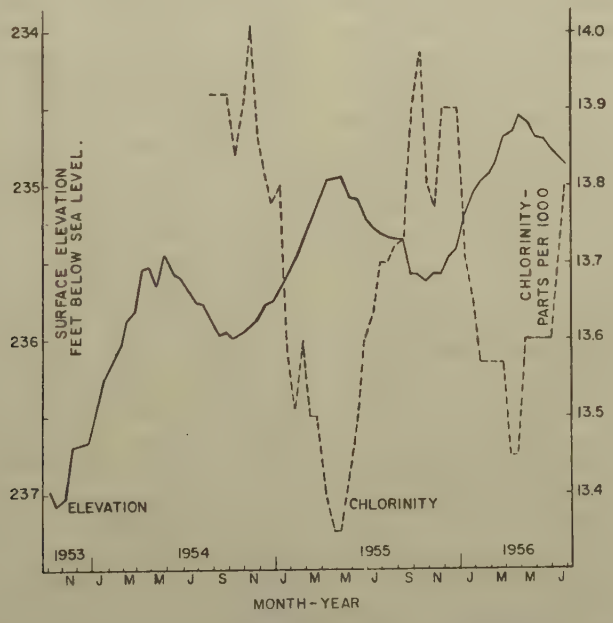

FIG. 2. Surface elevation at Fig Tree John (solid line) and chlorinity off Fish Springs (dotted line).

pletion of Boulder Dam more water was available, more land was put under irrigation, and more water entered the Sea whose level rose to −240 feet by 1948. It rose even more rapidly after 1948, due to land reclamation after World War II. By 1956 the surface elevation reached −234.4 feet, at which level the maximum depth is about 40 feet (12 meters).

In addition to the long-term changes in surface level, there is an annual cycle with highest elevation in spring and lowest in fall. Based on records from Figtree John's gauging station (maintained by the Coachella Valley County Water District), Figure 2 shows the cycle from 1953 to 1956. The cycle occurs because evaporation is low in winter, and exceeds water inflow only during the hottest part of the year. In addition, since 1935 the total annual inflow has exceeded the amount evaporated, resulting in a net increase in quantity of water in the Sea. Figure 2 indicates a net rise of one foot from November 1953 to October 1954, and a 0.4-foot rise from the fall of 1954 to the fall of 1955. These recent rises of level are less than the maximum yearly rise of 1.5 foot from 1951 to 1952, indicating that the rise of the Sea is slowing. The shape of the basin is such that with a rise in surface elevation the area of the Sea increases more rapidly than its volume (Holbrook 1927). Because of the increased area now available for evaporation, it is estimated, by the irrigation districts concerned, that, from the known rates of inflow of water and of evaporation, the level of the Sea may be stabilized at about the current surface elevation.

CHLORINITY

The salt content of the Sea shows an annual cycle with highest and lowest chlorinity occurring, respectively, at the lowest and highest surface elevations. Superimposed on Figure 2 is a plot of the chlorinity determinations made during the present study of the Sea. (Determinations were by titration of halides with silver nitrate.) From the data presented in Table 1, it is apparent that the weight ratio of Cl to total salinity (S) is not the same as in the oceans. Analyses since 1949 indicate that the ratio of Cl to S has ranged from 2.42 to 2.31, with an average value of 2.36. During the years of this study (1954–1956) the chlorinity in the main body of the Sea has ranged from 13.3 to 14‰, corresponding to a salinity range of from 31.4 to 33.0‰.

The main body of the Sea refers to the deeper portion and to the northern and western halves. As mentioned previously, most of the fresh water entering the Sea comes from the New and Alamo Rivers, which enter at the south (Fig. 1). The relatively uniform chlorinity of the main body of the Sea is illustrated by the following data. The chlorinity gradient from the north shore (outlet of Whitewater) to Fish Spring, a distance of about 7 miles, was from 13.6 to 13.9‰ on November 3, 1954. The previous week (October 26, 1954) samples from an east-west transect showed the chlorinity to be 13.7‰ on the east shore (Bombay Beach) and 13.8‰ on the west shore (off Truckhaven) with values of 13.9 and 13.8 in mid-sea. At a station on the south shore away from influence of stream discharge the average chlorinity of monthly samples from May 1955 to May 1956 was 12.9‰ with a range from 12.3 to 13.5. Chlorinity at this station (⁎3, Fig. 1, off the former mouth of the Alamo River) seems fairly typical of most of the southern and southwestern parts of the Sea.

In contrast to the relative uniformity and the small annual range of chlorinity in the main portion of the Sea, there are wide variations in chlorinity in the southeastern part where most of the freshwater enters. For example, data obtained November 8 and 10, 1954, illustrate the effect of freshwater entry at the southeast corner of the Sea, where the discharge of the Alamo River is eastward toward Mullet Island. A mile to the west of the river outlet the chlorinity was 13.9‰ at the bottom and 13.5 at the surface. A quarter of a mile east from the outlet, however, the chlorinity was 4.1‰, and at the west shore of Mullet Island it was 9.6. Two and one-half miles north of the island, along the shore of the Sea, surface chlorinity was 10.2, and 2 miles further north (about 4 miles from the mouth of the river) the surface value was 12.5 at shore, 12.9 a mile offshore, and 13.3 at the bottom (four meters). At Mullet Island (Station 4, Fig.

1) the average annual chlorinity was 10.8‰ with a range from 1.5 to 13.6 (a chlorinity of 1.5 is only twice that of the water in the Alamo River prior to entry into the Sea). The influence of fresh water has been noted as far north as Bombay Beach which is 9–10 miles from the river mouth. In general, the lower chlorinity is noticeable only along the southeast shore, and there is considerable delay before the effect of dilution is noticed in the main body of the Sea. Perhaps evaporation of the fresher water, which tends to float on the surface, modifies the dilution which then spreads slowly throughout the Sea by diffusion and a generally counterclockwise current.

Lowest chlorinity in the main body of the Sea during 1955 was only slightly below the lowest observed in 1956. It may be that the decrease in chlorinity, which has gone on since the peak of about 17.6‰ was reached in 1948–1949, has ended as the result of a new balance between evaporation and rate of inflow. If the surface elevation should stabilize at the current level, and if the amount of salt now entering remains the same, it can be calculated that the salinity of the Sea might be expected to increase again at a rate of about 0.4‰ per year.

TEMPERATURE

Although high temperatures are attained in summer, winter water temperatures are relatively low. The extreme range of temperature during a two-year period from August 1954 to August 1956 was from 10° to 36°C. Temperatures at the surface and at a depth of 3 meters were recorded by a dual-recording thermometer located on a dock at Sandy Beach where the maximum depth within reach was 3 meters. From these data were plotted the mean water temperatures shown in Figure 3. The figure includes mean air temperatures recorded at Fish Springs. No records were obtained for the energy of solar radiation, but the annual variation in the intensity of sunlight is shown by the increase in both air and water temperatures from a low in winter to a high in summer. From May to August the air is warmed more rapidly than the water: the mean air temperature consistently exceeds the mean water temperature. In June and July the water surface may be warmed so rapidly by solar energy that there is a significant difference between temperatures at surface and bottom, although the maximum depth of the Sea is only about 40 feet (12 meters). Table 2 shows subsurface temperatures taken with a reversing thermometer on selected days in 1955. From September to May temperatures were quite the same throughout the water column. At the end of May there was still only 0.7° difference between surface and bottom. On June 8, however, the difference was 5.3°, but this large difference was temporary and decreased to 2.2° by

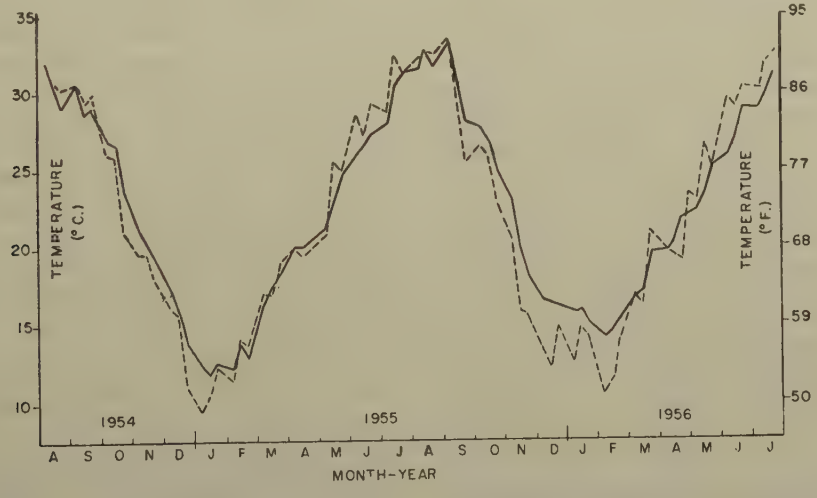

FIG. 3. Mean water temperature (solid line) at Sandy Beach and mean air temperature (dotted line) at Fish Springs.

June 14 and to 1° on June 30 (not shown). The difference in temperature remained slight through July 7, but on July 18 the surface was 3.9° warmer than the bottom. By the end of July the bottom temperature was up to nearly 30°, and surface and bottom differed by only 1.1°. During August the maximum observed difference was 0.8°, and at times surface and bottom temperatures were equal (as on August 24). Then from September on (until May) the usual difference in temperature between surface and bottom was 1° or less.

The temperature gradients that appeared during June and July were associated with changes in the weather. The warming of the surface on June 8 followed the appearance of the first protracted period without winds and with air temperatures above 38°. The warming recorded on July 18 followed four days without wind and with a mean air temperature of 40°. The appearance of a marked vertical temperature gradient seemed dependent on both rapid increase in surface warming by solar radiation and on lack of wind. Wind action mixes the shallow waters of the Sea rather thoroughly, as will be brought out in the next section dealing with concentrations of dissolved oxygen.

From Table 2 it should be evident that there is no thermocline of any duration. During the brief periods when surface and bottom temperatures are relatively far apart there may be a sharp transition between layers of water, but this may occur near the surface (between 2 and 3 meters on June 8), or at greater depth (between 7 and 9 meters on July 18), or there may be a uniform gradient (July 7). There is thus no permanent layering, but only temporary stratification following windless periods during early summer when the water temperatures are rising to their annual maximum. Once attained, the high water temperatures were maintained until mid-September. During this period (August and early September) the insolation of the water was consistently high as judged by air temperatures: the mean maximum air temperature for the entire month of August was 37.5°, and for the first 10 days of September the mean maximum was 40°.

TABLE 2. *Vertical distribution of water temperatures (°C) in 1955*

Depth m	May 27	June 8	June 14	July 7	July 18	July 27	Aug. 24	Sept. 15	Sept. 20	Oct. 19	Nov. 15
0	23.0	28.2	26.4	28.0	30.9	30.9	31.2	32.7	29.0	25.8	20.0
1		28.0		27.6							
2		27.5		27.0							
3		24.8	25.5	26.8	30.4						
5	22.4	24.0	25.0	26.6	30.0	30.2	31.3	31.8	28.2	25.5	19.8
7					29.7						
9		23.5			27.9						
11	22.3	22.9	24.2	26.6	27.0	29.8	31.2	31.6	28.2	25.6	19.9

During autumn the water cooled slowly: the bottom temperature did not fall below 30° until after mid-September; it then decreased to 25° in mid-October, and to 20° in mid-November. During this period of decreasing water temperatures the entire water column seemed to cool uniformly, without any great differences between surface and bottom comparable to the relatively large differences found during the period of increasing water temperatures during June and July.

DISSOLVED OXYGEN

Concentrations of dissolved oxygen were determined by the unmodified Winkler method. The application of this technique to sea water is discussed by Jacobsen *et al.* (1950).

Near shore there are great diurnal changes in oxygen concentration. For example, in water with a depth of about 3 meters approximately 100 meters off shore on September 11, 1954, surface concentrations ranged from 4.0 mg/L at 6:40 A. M. to 8.7 mg/L at 6:20 P. M. Bottom concentrations at these hours were 3.3 and 8.8 mg/L, respectively. Water temperatures which ranged from 29.2° at dawn to 31.4° at sunset were then at about their yearly maximum. The evening concentration of 8.7 mg/L is well above the saturation value for the high temperature concerned.

During the colder part of the year the surface water may become even more supersaturated with oxygen. Thus on February 17, 1955, when the water temperatures were 15–16°, the evolution of oxygen by an early bloom of phytoplankton at a station approx-

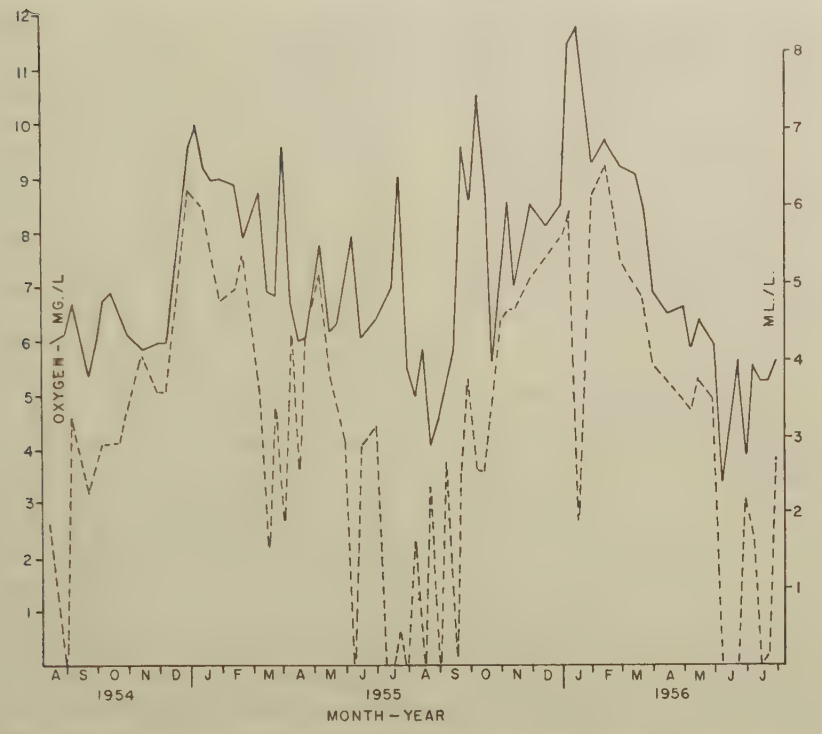

FIG. 4. Dissolved oxygen at mid-day: surface (solid line) and bottom (dotted line) three miles off shore from Fish Springs.

imately one mile offshore above a depth of seven meters raised the concentration of dissolved oxygen from 8.8 mg/L at dawn to 11.5 mg/L at noon. The bottom concentrations (seven meters) ranged from 5.3 to 5.7 mg/L.

As representative of the concentrations of dissolved oxygen in the main body of the Sea, Figure 4 shows values obtained from mid-day samples taken at approximately weekly intervals during 1955 and semi-monthly during 1956 at a station about three miles east of Fish Springs in the deepest part of the Sea (about 12 meters). Mid-day surface concentrations ranged from a maximum of 11.8 mg/L on January 16, 1956, to a minimum of 3.4 mg/L on June 6, 1956. Bottom values ranged from such high winter concentrations as 8.9 mg/L on January 4, 1955 and 9.3 mg/L on February 14, 1956, to the low of zero, which was recorded on several occasions in summer.

In general, during winter there are high concentrations of dissolved oxygen throughout the water column. However, as early as January, 1956, and during March and April of 1955 there were times when concentrations were much lower at the bottom. In early June of both 1955 and 1956 the bottom became anoxic, but the condition did not last long. Figure 4 indicates the temporary nature of the bottom oxygen depletion. In July of 1955, however, only one of six determinations showed any oxygen present, and that one (on July 27) showed only 0.65 mg/L. Periods when oxygen is absent at the bottom are periods without wind. The surface of the sea becomes glassy and so smooth it mirrors the surrounding hills. One such windless period occurred in mid-July of 1955. Records of samples taken at 9:30 A. M. on July 12, at noon on July 18, and at 6 A. M. and 6 P. M. on July 19 are quite similar in showing no oxygen present at depths below 9 meters. Morning and evening temperatures and pH, as well as the concentrations of dissolved oxygen found on July 19 are shown in Table 3.

In contrast to these records from the

TABLE 3. *Early morning and evening concentrations of dissolved oxygen, temperature, and pH, 3 miles offshore from Fish Springs on July 19, 1955*

Depth m	6 A. M.			6 P.M.		
	Diss. O_2 mg/L	Temp. °C	pH	Diss. O_2 mg/L	Temp. °C	pH
0	7.4	30.6	8.69	9.3	31.4	8.73
7	6.4	29.4	8.55	5.6	30.3	8.58
9	0	28.0	8.34	0		8.37
11	0	27.4	8.34	0	27.5	8.26

TABLE 4. *Evening and early morning concentrations of dissolved oxygen, temperature, and pH, 3 miles offshore from Fish Springs, mid-September and mid-October, 1955*

Depth m	September 15, 5 P.M.			September 16, 5:45 A.M.		
	Diss. O_2 mg/L	Temp °C	pH	Diss. O_2 mg/L	Temp °C	pH
0	10.1	32.7	8.74	0.8	30.4	8.28
5	3.4	31.8	8.56	0.7	30.4	8.26
11	0.5	31.6	8.52	0.6	30.6	8.3
	October 18, 4 P.M.			October 19, 6 A.M.		
0	8.9	25.5	8.7	3.1	25.0	8.48
5	3.7	25.6	8.52	3.1	25.2	8.48
11	2.6	25.6	8.48	3.1	25.3	8.44

windless period in mid-July where there was little difference between morning and evening concentrations, records for mid-September and mid-October (Table 4) show the disappearance of surface to bottom differences in concentrations of dissolved oxygen (as well as in pH and temperature) during the 12-hour period between samplings. The uniformity of the water column in the early morning seems to indicate that, in addition to the effect of overnight respiration by plankton, there was mixing due to wind action. During 1956 the bottom anoxia again appeared during June, but was absent in early July. Then, after a windless period, by July 16 the condition was as indicated in Table 5 with data from samples taken at 10 A. M. Ammonia, sulfide, and pH, included in the table, will be discussed later. Of interest here is the absence of oxygen below 8 meters, and the three-degree difference in temperature between surface and bottom. On July 18, two days later and after a day with strong south wind, the data in Table 6 were obtained in the same location, again at 10 A. M. The thorough mixing is indicated by the similarity of surface and bottom temperatures, the oxygen at the bottom, the small difference in pH, etc.

Following a period of oxygen depletion at the bottom, oxygen concentrations at the surface may be lowered temporarily, as at dawn on September 16, 1955, when the concentration at the surface three miles from shore was only 0.8 mg/L (at the bottom it was 0.55 and at mid depth 0.7 mg/L). The biological effect of another period of oxygen depletion (data shown in Table 5) is indicated by a field note made July 16, 1956: "Fish 'kill' general throughout the north end of the Sea. A few dead *Bairdiella* floating at surface about a mile offshore from Fish Springs. Many dead on beaches July 13 to July 16." (The *Bairdiella* referred to is a croaker, the most prevalent fish in the Sea.) Another example of the biological effect of the anoxia is the disappearance of *Neanthes succinea*, a bottom-dwelling worm, from the bottom at depths below about 8 meters during summer.

It seems that only the water below about

TABLE 5. *Dissolved oxygen, temperature, pH, ammonia, and sulfide, 3 miles offshore from Fish Springs, 10 A.M., July 16, 1956*

Depth m	Diss. O_2 mg/L	Temp. °C	pH	NH_3-N µg-at./L	Sulfide-S µg-at./L
0	5.4	30.0	8.51	10	0.35
6	4.3	29.0	8.42	11	0.7
8	0.5	28.4	8.35	22	1
9	0		8.19	35	15
10	0	27.2	8.16	40	38
11	0		8.08	48	64
12	0	27.0	8.02	36	85

TABLE 6. *Dissolved oxygen, temperature, pH, ammonia, and sulfide 3 miles offshore from Fish Springs, 10 A.M., July 18, 1956*

Depth m	Diss. O_2 mg/L	Temp. °C	pH	NH_3-N µg-at./L	Sulfide-S µg-at./L
0	5.0	29.5	8.50	17	0.1
6	4.5	29	8.49	15	0.1
8	4.4	29	8.47	12	0.1
10	3.7	29	8.42	14	0.1
12	3.4	29	8.40	17	0.1

8 or 9 meters becomes strongly anoxic. The region concerned thus includes the central, deepest part of the Sea, in approximately the area below the −265 foot contour shown in Figure 1. Regions near shore with depths less than 8 or 9 meters do not seem to be affected. Thus, at dawn on September 16, 1955, when the surface concentration 3 miles from shore was only 0.8 mg/L, the concentration 100 meters from shore was 5.5 mg/L both at the surface and at the bottom (3m).

HYDROGEN ION CONCENTRATION

Determinations in the field with a Beckman Model G portable pH meter showed pH to range from 8.3 to 8.6 in winter and from 8.5 to 8.8 in summer. The water of the Salton Sea contains a different ratio of carbonate to bicarbonate than the oceans, and the pH is higher. As in the oceans, photosynthetic uptake of carbon from the buffer system causes a diurnal cycle of pH. An example (Table 7) of the diurnal range of pH near shore and one mile offshore on April 12, 1955, illustrates the daily trend during a time of year when water temperatures were below 20°C.

Data from the same locations on July 19, 1955, (shown in Table 8) illustrate the pH values found during the hottest time of the year, on a day when surface water temperatures at shore were 31.4° at dawn and 31.6° at sunset.

Data for vertical distribution of pH and the diurnal range observed in the deeper part of the Sea on the same date (July 19, 1955) are included in Table 3. Tables 4 and 6 also include data for pH values in deeper water.

The pH of the bottom mud is more acid

TABLE 7. *Diurnal changes in pH and temperature, April 12, 1955*

	Near shore				One mile offshore			
	Surface		Bottom (3 m)		Surface		Bottom (7 m)	
	pH	temp.	pH	temp.	pH	temp.	pH	temp.
6 A. M.	8.32	19.2	8.39	19.0	8.41	18.8	8.39	18.6
12 M.	8.50	21.0	8.40	19.8	8.56	21.0	8.51	19.2
6 P. M.	8.62	21.2	8.60	20.5	8.58	20.0	8.51	18.8
Midnight	8.47	19.4	8.47	19.2	8.46	18.9	8.40	18.2

TABLE 8. *Diurnal changes in pH and temperature, July 19, 1955*

	Near shore				One mile offshore			
	Surface		Bottom (3 m)		Surface		Bottom (7 m)	
	pH	temp.	pH	temp.	pH	temp.	pH	temp.
5 A. M.	8.67	31.4	8.63	31.4	8.62	31.2	8.52	29.7
11 A. M.	8.74	31.4	8.72	31.4	8.7	31.0	8.62	29.2
6 P. M.	8.81	31.6	8.81	31.6	8.75	31.5	8.63	30.3

than that of the water. During anoxic periods in 1956, pH and redox potentials of the mud were measured. The pH of the bottom mud was 7.5 on July 6 and 7.4 on June 22. The oxidation and reduction potential of the mud recorded on these days was −474 mV and −480 mV, respectively.

SULFIDE

The depletion of oxygen at the bottom seems to be due to bacterial decomposition of a thick layer of plankton that accumulates by early summer. On the surface of this organic layer the sulfur bacterium *Beggiatoa* was noted in September and October 1955. The presence of sulfur bacteria and of periods of bottom anoxia during the summer and fall of 1955 led to interest in the possibility that high concentrations of sulfide might occur when oxygen was absent. A method of determing sulfide in sea water was obtained from Dr. N. W. Rakestraw of the Scripps Institution of Oceanography. The method, which is a modification of the Standard Methods colorimetric technique utilizing para-amino-dimethyl analine and ferric chloride to produce methylene blue in the presence of sulfide ion, proved satisfactory in the Sea.

Beginning in January 1956 and continuing through June determinations were made at approximately semi-monthly intervals; six samples were analyzed during July. The average value of 12 bottom samples (from a 12-meter depth) taken from January through May was 0.25 μg-at./L with a range from 0 to 0.5. On June 6, the day of the first appearance of oxygen depletion at the bottom, the concentration of sulfide rose to 1.5 μg-at./L at the bottom, and on June 22 the concentration was 50 μg-at./L. In early

July concentrations were low: 0.5 μg/at./L on both July 2 and 9. Then on July 16 the bottom concentration of sulfide rose to 85 μg-at./L. The bottom values for the last three determinations made in July were 0.1, 1.0, and 0.6 μg-at./L on July 18, 24, and 31, respectively. In contrast to the high values and the great variation in concentrations at the bottom, the average sulfide concentration in 20 surface samples analyzed during the 7 months from January through July was 0.3 μg-at./L with a range from 0 to 0.6 μg-at./L. Data on vertical distribution of sulfide on July 16 and 18 are included in Tables 5 and 6.

AMMONIA, NITRATE, AND PHOSPHATE

Ammonia was determined by direct Nesslerization after precipitation of interfering ions by the Witting-Buch technique (Wirth and Robinson 1933), nitrate by the diphenylbenzidine method (Atkins 1932), and phosphate by the molybdate-stannous chloride method (Wooster and Rakestraw 1951). It should be pointed out that, of these colorimetric methods used for analysis, only the one for phosphate is well-established. The method used for ammonia proved satisfactory because of the high concentrations present, but the method for nitrate was inconsistent, and the nitrate concentrations given below are of questionable value.

TABLE 9. *Average concentration and range of concentration of ammonia, nitrate, and phosphate in μg-at./L.*

Station Number	Depth m		Ammonia	Nitrate	Phosphate
1	0	Average	5.35	0.85	0.47
		Range	0.2–13.5	0.2–1.9	0.33–1.0
	6	Average	5.9	0.8	0.61
		Range	0.2–18.3	0.2–1.73	0.32–1.95
	12	Average	9.5	0.78	0.45
		Range	0.4–40.0	0.2–1.85	0.3–0.85
2	0	Average	5.5	0.91	0.47
		Range	0.1–12.3	0.4–2.0	0.3–0.6
	3	Average	5.9	0.99	0.51
		Range	0.2–13.8	0.4–2.2	0.2–0.85
3	0	Average	4.8	0.93	0.71
		Range	0.1–10.0	0.4–1.4	0.45–1.1
4	0	Average	12.3	7.63	1.1
		Range	0.2–30.0	0.8–30.0	0.55–4.2

Table 9 summarizes data obtained at four stations. Data from Stations 1 and 2 are based on weekly samplings during a two-year period (Sept. 1954 to July 1956). Station 1 is representative of the deepest water in the Sea (depth 12 meters) located about 3 miles offshore from Fish Springs (see Fig. 1). Station 2 is representative of the conditions at shore in the main part of the Sea, located about 100 meters offshore from Fish Springs. Data from Stations 3 and 4 are based on monthly samplings during a one-year period (May 1955 to May 1956). Station 3 is on the south shore near the former mouth of the Alamo River (depth about 1.5 meters), a location relatively little influenced by present stream discharge. Station 4 is off Mullet Island (depth also about 1.5 meters), about a mile from the present mouth of the Alamo, a location where the effects of stream discharge are noticeable. Because the water was so shallow at Stations 3 and 4 only surface samples were taken.

In the main body of the Sea (Stations 1 and 2) the range of concentrations of nitrate-N was nearly the same at all depths; the average was slightly higher at the surface and near shore. The range and average concentration of phosphate-P was greatest at mid-depth, while ammonia concentrations were highest at the bottom.

Concentrations of ammonia showed a marked seasonal trend in the main body of the Sea. Figure 5 shows the trend at both surface and bottom at Station 1. Concentrations at the bottom rose from a low of 0.4 μg-at./L in winter to about 10 μg-at./L by May, and then reached a maximun above 30 μg-at./L during summer. Concentrations fell to 10 μg-at./L in September and October and then continued downward to the winter low. In contrast to ammonia, there were only slight differences between surface and bottom concentrations of nitrate and phosphate (only the bottom values are shown in Fig. 5), and no significant seasonal trends are indicated.

At Station 3 (the south shore of the Sea) there were only slightly higher concentrations of phosphate and nitrate, and slightly lower concentrations of ammonia than were found in the main body of the Sea. How-

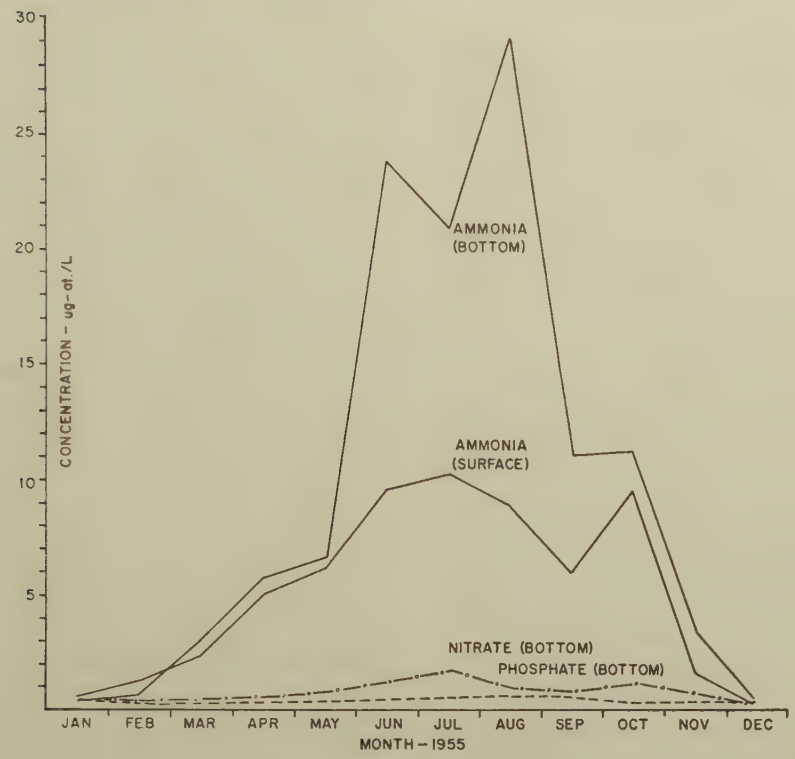

FIG. 5. Ammonia-N, nitrate-N, and phosphate-P three miles off shore from Fish Springs.

ever, at Station 4 (off Mullet Island and in line with the discharge of the Alamo River) there were significantly higher concentrations of nitrate and phosphate than at any of the other stations. The highest average concentration of ammonia also occurred at Station 4, although the maximum there was exceeded by the maximum found at the bottom in deeper water (at Station 1).

The concentrations of ammonia, nitrate, and phosphate at Station 4 depend on the quantity of water discharged into the Sea, which varied depending on seasonal differences in irrigation practice in the Imperial Valley. Most of the water "wasted" into the Sea has been used for irrigation, and it carries salts, fertilizer, and soil from the land. In the water of the New and Alamo Rivers near their points of entry into the Sea the average concentration of phosphate was 5 µg-at./L, and these rivers carried an average of 10 µg-at. of ammonia and 25 µg-at. of nitrate per liter. The concentrations of these plant nutrients at the surface in the main body of the Sea are, of course, much lower. Thus, the amount of dissolved inorganic phosphate at the surface (Station 1) averaged 0.4 µg-at./L, which is about 1/10 the concentration present in the river water. And the amount of nitrogen momentarily available at the surface (Station 1) averaged about 6 µg-at./L (present largely as ammonia) in contrast to the average of 35 µg-at. of available nitrogen (mostly in the form of nitrate) present in the river waters. Most of the nitrogen and phosphate in the Sea are not, of course, in the dissolved inorganic form, but are assimilated by the organisms present and become incorporated in them and in the organic mud of the bottom. The Sea is highly productive and there seems to be an effective cycle of use and re-use of the nutrients that enter. The organisms in the Sea and their productivity will be discussed in detail elsewhere, but the following section summarizes some of the biological aspects of the Salton Sea.

DISCUSSION

The Salton Sea is of fresh water origin but due to a high rate of evaporation its waters now have a total salt content comparable to that of the oceans. Although the difference in proportion of ions might be expected to make the Sea a difficult environment for marine organisms, the fauna of the Sea consists of hardy marine animals. The flora, too, is predominantly marine. The organisms living in the Sea must be able to tolerate a great range of temperature (great for a marine environment) and a high summer temperature; in addition they must also tolerate the relatively large diurnal fluctuations in pH and in concentrations of dissolved oxygen that result from photosynthesis and respiration. However, the diurnal changes would seem to be minor stresses compared to the greater stress occurring annually when decomposition of settled zoo- and phyto-plankton brings about periods of oxygen depletion at the bottom. The concurrent high concentrations of sulfide and ammonia also contribute toward making summer the most difficult season for many organisms to survive.

Marine plants and animals without wide ranges of tolerance from the normal marine environment do not survive in the Sea. Thus, there are no macroscopic red or brown algae, for example, and filamentous green algae are limited to the fresh water inlets. The only macroscopic plants in the Sea are blue-green algae which form conspicuous growths on pilings and on the bottom in shallow areas. The growths of blue-green algae occasionally break free from the bottom, and float at the surface. Although the blue-green algae are thus conspicuous at times, the predominant plants of the Sea are dinoflagellates, diatoms, and other single-celled algae which live suspended in the water.

The dinoflagellates include a species of *Exuviella*, similar to *baltica*, which was absent from collections only during August and September. It, and another dinoflagellate (a species of *Gymnodimium*?), were most numerous during sporadic local blooms near shore where they appeared at times in such numbers that they colored the water.

Of the diatoms of the Sea, *Cyclotella caspia* is perhaps the most representative, being present in all collections. Maximum numbers occurred in spring and fall. A pennate diatom, *Nitzschia longissima*, although absent (or present in small numbers) during summer, was the most abundant plant cell present in fall and winter.

Other dinoflagellates and diatoms, as well as silicoflagellates, coccolithophores, a euglenoid, etc., occur in the Sea, but none attain populations as great as the species mentioned above. One other plant cell deserves mention because it is the most prevalent plant of the phytoplankton in early summer. It is a small green alga, resembling *Westella botryoides*, whose identity has not been confirmed.

Plant cells of the types described above serve as food for the zooplankton which is limited to four invertebrate animals of numerical significance. The most abundant animal in the summer plankton is a rotifer, *Brachionus plicatilis* Müller, which is present from May-June to December-January. Maximum numbers occur in August. The taxonomic help of Dr. E. H. Ahlstom, who has monographed the genus *Brachionus* (Ahlstom 1940), is gratefully acknowledged.

Neanthes succinea (Frey and Leuckart), a nereid worm, was introduced into the Sea in 1930 and first reported prevalent in 1936 (Hartman 1936). The adults live in the bottom mud and spawn at the surface. The larvae have a planktonic period of about two weeks. Based on plankton sampling, it would seem that *Neanthes* has two peaks of spawning, March-June, and October-November, but that spawning occurs the year around, at least near shore where eggs were found every month of the year. In deeper water (below 8–9 meters) *Neanthes* disappears from the bottom during summer, and neither eggs nor larvae were found in the plankton collected above such depths during August and September. It seems that the adults at the bottom are killed by lack of oxygen (or by high sulfide or ammonia?) during anoxic periods. Only after oxygen becomes present continuously again in fall does the central, deepest part of the Sea become repopulated with *Neanthes*.

Barnacles find only limited space for attachment, because rocks occur only in the volcanic plugs at the southern shore of the Sea, but *Balanus amphitrite* Darwin covers submerged vegetation and debris, pilings, etc. The barnacle was probably introduced during the 1940s and was first reported from the Sea by Rogers (1949). Immature states of *Balanus* appeared in all plankton collections with the exception of a few collections in January-February and some in July. Peaks of spawing occurred March-June and August-September. Greatest numbers were found in April.

The life cycle of *Neanthes*, and the rate of growth and of settling of *B. amphitrite* in the Sea have been studied by Richard H. Linsley, whose results will be reported elsewhere.

A copepod, *Cyclops dimorphus* Kiefer, was redescribed from the Sea by Johnson (1953). It is present in the plankton only during the warmest months of the year. It disappears from the plankton in December-January and reappears in June-July. Maximum numbers occur in autumn.

Most of the plankton animals are not eaten directly; the fish present in the Sea do not include any species which, as adults, feed by screening the plankton. Some of the plankton animals are eaten by young stages of fish, but the majority accumulate, together with the settled phytoplankton, to form the rich bottom mud on which *Neanthes* feed. Studies by Jay C. Quast indicate that the adult worms are the principal fish food of the Sea.

Fish are the final link in the food chain within the Sea. There are three species of minor importance that live along the shores; one, *Cyprinodon macularius*, is native, the other two, *Gambusia affinis* and *Gillichthys mirabilis*, were introduced. The two principal species present, *Bairdiella icistius* and *Cynoscion* sp., have been studied by Richard R. Whitney. The Gulf Croaker, *Bairdiella*, was introduced in 1950–51 from the Gulf of California. Although many species of fish have been introduced, only *Bairdiella* has built up great numbers: it was able to establish itself because of the presence of *Neanthes*, which had been introduced 20 years before. (The situation seems similar to the introduction of *Neanthes* as a fish food in the Caspian Sea, reported by Zenkevich 1951.) With *Bairdiella* established there is food for a predatory fish, and one of the species of *Cynoscion* (white sea bass) that were introduced almost yearly from 1950 to 1955 has spawned in the Sea. It may be that *Cynoscion* will establish itself and thus add another link to the food chain.

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A Volumetric Study of Zooplankton Distribution in the Cape Hatteras Area[1]

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ABSTRACT

The displacement volumes of 82 zooplankton samples, collected from the offshore waters of North Carolina during May and June of 1953 and January 1954, are determined and reported. A brief review is given of the topographic and hydrographic features of the area. A comparison of zooplankton volumes is made with other areas in the eastern and western North Atlantic. In the present area, the mean zooplankton volume in May was 0.29 cc/m^3; in June, 0.28 cc/m^3; in January, 0.20 cc/m^3. The volumes of zooplankton are shown to be least in shallow coastal areas and in offshore Florida Current water and to be generally at a maximum near the mid-shelf region.

Winter volumes in the present area were about 70% of the late spring values, while north of Cape Hatteras the plankton undergoes a 50% reduction between these seasons. Over Georges Bank the winter volumes are about 15% of the spring volumes, and in Norwegian waters the winter plankton volume is less than 3% of the spring value. The relatively slight reduction in plankton volumes from spring to winter in the Cape Hatteras area appears due to (1) the proximity of the Florida Current which supplies the shelf waters with reproducing populations of oceanic plankton species, and (2) the relatively high winter water temperatures and high solar radiation which insure a moderate standing crop of phytoplankton and hence a food supply for the animal fraction of the plankton.

INTRODUCTION

The zooplankton volumes reported in this study represent the first quantitative collections of plankton from the shelf waters off North Carolina south of Cape Hatteras. Although only two cruises and a total of 82 samples are involved, it is desirable that the plankton data from this "new" area be made available in the literature. The distribution of zooplankton volumes in the present region is described in some detail, first, to show the relationship between the distribution of volumes as influenced by the various water bodies in the area, and second, to permit an adequate comparison of zooplankton volumes in this area with some other areas from which quantitative data are available. The copepod fraction of these samples was analyzed and occasional reference is made to the abundance and distribution of this group. A detailed analysis of the copepod fraction will be the subject of a subsequent paper.

[1] Based on a dissertation submitted in partial fulfillment of the requirements for the doctoral degree at Harvard University 1956.

METHODS

The zooplankton samples were collected by personnel of the Woods Hole Oceanographic Institution and were made available to the author by Mr. Dean F. Bumpus of that organization. The location of the area under study is shown in Figure 1. The collection is represented by 82 samples taken at 54 stations in May and June 1953 (Fig. 2) and in January 1954 (Fig. 3). The stations were occupied along 8 sections, each at approximately right angles to the shore line. At most of the stations of the spring cruises the upper and lower halves of the water column were sampled with separate nets. The deeper net sampled water from near the bottom at most of the shelf stations. In the January cruise only the water over the outer half of the shelf was sampled with such divided tows, the remaining stations consisting of single hauls from near the bottom to the surface. In Figures 2 and 3 the open circles represent hydrographic stations only.

The samples were collected with Clarke-Bumpus plankton samplers (Clarke and Bumpus 1950) fitted with a number 2 silk

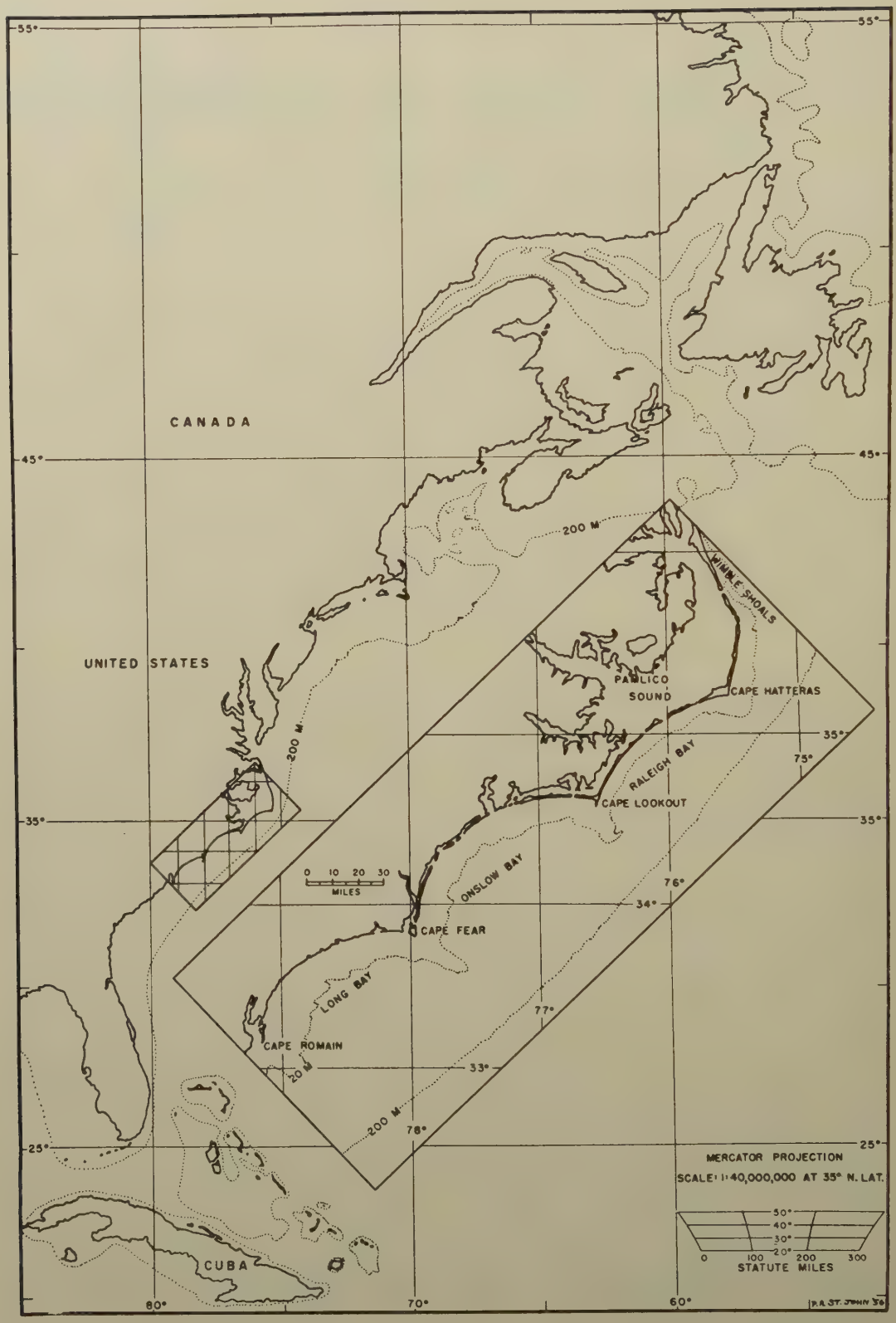

FIG. 1. Chart of the east coast of the United States with inset of the area under consideration.

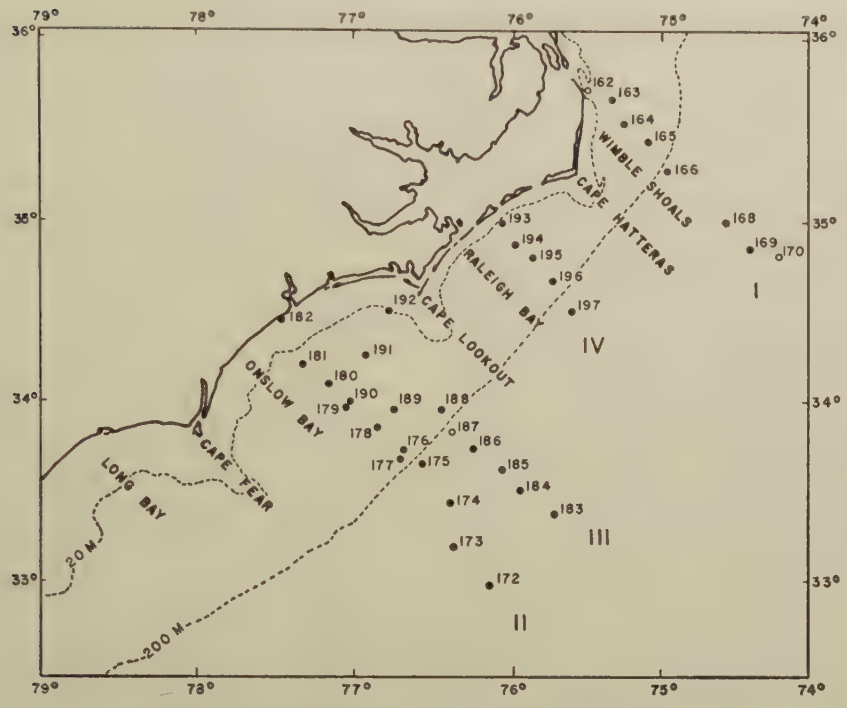

FIG. 2. Location of stations, May and June, 1953.

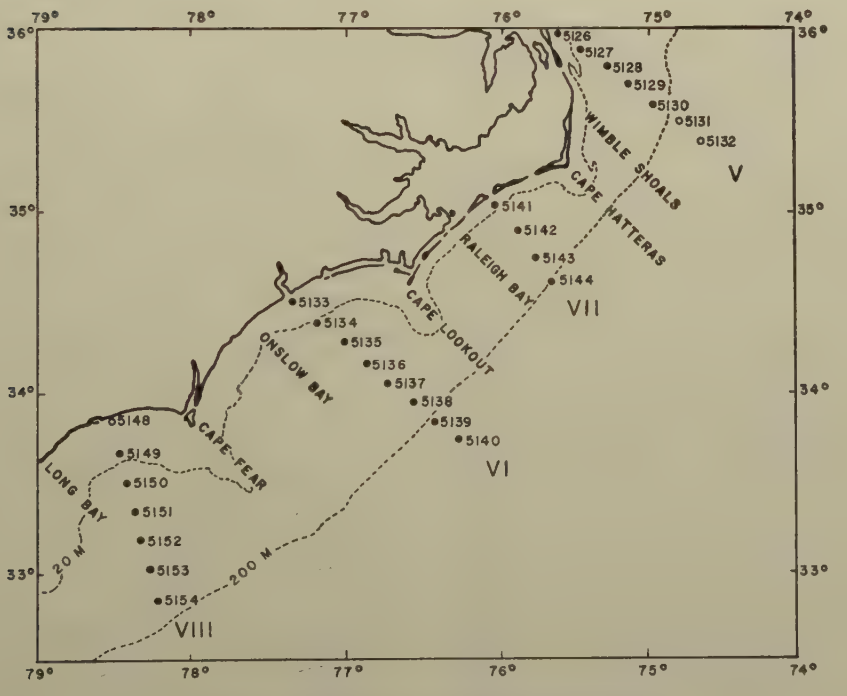

FIG. 3. Location of stations, January, 1954.

TABLE 1. *List of stations and related data for all cruises*

Station	Depth range of oblique tows m	Water filtered m^3	Volume of zooplankton less Chaetognatha cc/m^3
163*	3	9.4	0.03
164†	1	212.0	0.01
165	15–0	7.1	0.64
166	40–0	7.1	0.36
166	190–0	9.7	0.20
168*	9	0.9	0.03
169*	9	14.0	0.25
172	75–0	20.7	0.05
172	150–75	31.8	0.05
173	80–0	24.4	0.12
173	160–80	30.0	0.00
174	80–0	20.6	0.14
174	160–80	27.8	0.07
175	90–0	5.1	1.90
175	180–90	12.2	0.00
176	30–0	12.2	0.34
176	62–30	16.3	0.20
177	50–0	13.8	0.18
177	100–50	28.7	0.10
178	15–0	15.8	0.40
178	30–15	16.8	0.49
179	31–0	18.0	0.55
180	15–0	14.1	0.31
180	30–15	16.1	0.30
181	20–0	15.2	0.19
182	12–0	7.3	0.01
183	80–0	23.1	0.12
183	160–80	29.1	0.06
184	70–0	23.5	0.06
184	140–70	29.7	0.06
185	65–0	29.7	0.13
185	130–65	33.2	0.07
186	50–0	33.7	0.09
186	100–50	38.5	0.08
188	20–0	8.3	0.29
188	40–20	9.7	0.51
189	20–0	8.4	0.35
189	40–20	12.4	0.37
190	16–0	7.0	0.42
190	32–16	9.8	0.30
191	13–0	8.1	0.27
191	26–13	13.5	0.22
192	10–0	13.5	0.10
192	20–10	14.0	0.03
193	20–0	11.3	0.44
194	10–0	11.0	0.18
195	15–0	11.4	0.09
195	30–15	8.9	0.50
196	30–0	9.0	0.11
196	60–30	27.0	0.29
197	80–0	13.1	0.35
197	160–80	15.8	0.01
5126	20–0	15.4	0.19
5127	20–0	14.1	0.42
5128	28–0	15.9	0.12
5129	45–0	16.7	0.12
5130	25–0	16.5	0.30
5133	13–0	11.8	0.12
5134	19–0	18.5	0.21
5135	25–0	15.2	0.06
5136	28–0	12.0	0.15
5137	39–0	20.4	0.19
5138	20–0	13.2	0.30
5138	40–20	14.7	0.16
5139	50–0	15.6	0.25
5139	100–50	12.9	0.08
5140	50–0	18.1	0.01
5140	100–50	19.2	0.00
5141	15–0	11.5	0.26
5142	16–0	10.8	0.01
5142	24–12	16.9	0.00
5143	20–0	16.1	1.08
5143	40–20	17.8	0.44
5144	50–0	19.3	0.05
5144	100–50	20.8	0.09
5149	15–0	5.9	0.17
5150	20–0	11.3	0.01
5151	24–0	13.3	0.04
5152	30–0	13.7	0.17
5153	30–5	16.8	0.35
5154	50–0	15.5	0.15
5154	100–50	22.6	0.36

TABLE 1. *Continued*

* Horizontal tows.

† Non-recording conical net. Volumes estimated.

net and towed "obliquely" through the vertical distance of the water column. The average length of time of the tows was 33 minutes, ranging from 10 minutes to one hour. Volumes of water filtered by the samplers averaged about 16 m^3 and ranged from 0.9 m^3 to 38.5 m^3. During the May cruise fourteen horizontal surface tows were made with a non-recording, ½-meter net also of number 2 silk. Since this latter net does not record the volume of water filtered while towing, all but one of these surface tows have been omitted from the calculations, permitting a more accurate comparison of zooplankton volumes from station to station. The surface tow at Sta. 164 is included since this tow represents the only haul at that station. The omission of the remaining surface tows does not represent a serious loss of data since the shallower tow of a divided haul (or the single tow at stations where only one sample was collected) invariably sampled the surface

layers. All samples were preserved in 5% formalin.

Volumes were determined by displacement according to the procedure of Wiborg (1954). These volumes are reported as cubic centimeters of plankton per cubic meter of water (cc/m^3). The Chaetognatha had been removed from these samples for separate study (Bumpus and Pierce 1955), and the volumes determined here do not include this group. The chaetognaths in these samples averaged about 10 mm by 0.8 mm and displaced a volume of about 5 mm^3 (E. L. Pierce, private communication). The chaetognaths formed about 12% of the average zooplankton volume in May, about 9% in June, and about 12% in January. A list of stations, depth ranges of the tows, and volumes of each sample are given in Table 1.

TOPOGRAPHY OF THE AREA

South of Cape Hatteras the edge of the continental shelf (the 200-meter depth contour) does not follow the cuspate form of the coastline with the result that the shelf varies in width from about 55 miles off Cape Fear to about 20 miles off Cape Hatteras (*cf.* Fig. 1). The continental shelf broadens to the north and south of North Carolina so that the 200-meter line lies about 110 miles off New York City and about 100 miles off Brunswick, Georgia.

Beyond the edge of the shelf the continental slope (depth range 200 to 2000 m) has a relatively uniform width, averaging about 15 miles north of Cape Lookout. South of this Cape, however, the slope broadens until, at its widest point off Jacksonville, Florida, the distance between the 200 and 2000 meter lines is almost 300 miles.

HYDROGRAPHY OF THE AREA

The present samples were collected from three distinct bodies of water, *viz.*, the Florida Current, Virginian coastal water, and Carolinian coastal water. The latter two terms were introduced by Bumpus and Pierce (1955) to designate the waters over the continental shelf north and south of Cape Hatteras, respectively. During the winter months Carolinian and Virginian coastal waters ordinarily are separated by a strong horizontal temperature gradient which Parr (1933) has termed the "temperature barrier." A brief review follows of the hydrographic data collected on these cruises. The reader is referred to Bumpus and Pierce (1955) for the detailed account of these data.

The Florida Current. Water of the Florida Current flows in a northeastward direction off the North Carolina coast with maximum velocities at its axis in excess of 150 cm/sec. In general this current parallels the 200-meter depth contour south of Cape Hatteras. The approximate axis of the current during mid-May of 1953 was about 18 miles southeast of the 200-meter line off Onslow Bay (von Arx, Bumpus, and Richardson 1955), and as the stream follows this depth contour northeastward its axis approaches very close to shore at Cape Hatteras.

Salinity of the shallower layers of the Florida Current off North Carolina is near 36.0‰ or higher during the winter and spring months of the year, and temperatures near the surface increase from about 20°C in January to over 25° in the late spring. Bumpus (1955) has shown that the Florida Current south of Cape Hatteras changes its direction of flow so that its waters may penetrate over the adjacent continental shelf, either along the bottom or at all depths.

Virginian Coastal Water. From Cape Cod southward to Cape Hatteras the winter surface temperatures of the coastal water were found generally to range from less than 2° near the coast to about 8° over the 200-meter depth contour (Bigelow 1933). A few miles north of Cape Hatteras, Virginian coastal water temperatures are near 5° inshore, and over the 200-meter line are above 10°. By May and June vernal warming has increased water temperatures over the entire shelf from about 12° at Cape Cod to over 20° a few miles north of Cape Hatteras. During the present cruises, surface temperatures just north of Cape Hatteras in January were about 8° near the coast, and 16° over the edge of the shelf. Surface temperatures in May were

more than 17° near the coast, and 22° over the 200-meter line.

Winter salinities of the coastal water north of Cape Hatteras vary in inshore regions from 32‰ near Cape Cod to about 30‰ just north of Cape Hatteras. Salinities over the edge of the continental shelf are near 34‰ (Bigelow and Sears 1935). Vernal warming of adjacent land masses results in the melting of winter snows, and consequent water drainage decreases salinities to below 32‰ in coastal areas and to about 33‰ over the edge of the shelf. During the present January cruise surface salinities in the vicinity of Cape Hatteras ranged from about 31‰ inshore to about 34‰ over the 200-meter line. The data collected near Cape Hatteras in May show salinities less than 32‰ next to the coast, increasing to over 36‰ near the edge of the shelf. This latter salinity value represents a direct influence of the Florida Current.

Carolinian Coastal Water. Bumpus (1955) and Bumpus and Pierce (1955) have shown that during the winter months for which data are available water temperatures south of Cape Hatteras range from about 10° in coastal regions to over 20° near the edge of the shelf. Salinity values of less than 34‰ may occur next to the coast in Onslow and Long Bays, and the 36‰ isohaline may be found well inshore of the edge of the shelf. In the present January cruise, this isohaline had penetrated to within 12 miles of the coast in Onslow Bay.

During the spring months surface temperatures south of Cape Hatteras are increased to near 25°, and salinities are maintained generally near 35‰ or slightly higher. The hydrographic data collected on these cruises show that temperatures and salinities of Carolinian coastal water only rarely reach the low values common to Virginian coastal water to the northeast. The differences are due to the reduced freshwater addition south of Cape Hatteras (Bumpus 1955) and to the proximity of the Florida Current which carries warm, highly saline water from more southern latitudes.

ZOOPLANKTON VOLUMES

The distribution of zooplankton volumes is plotted for each of the eight sections in Figures 4 through 11. Where the water column at a station was sampled with a divided tow the zooplankton volume of each tow was averaged and the resulting value represents the mean volume of the zooplankton for the water column as a whole. The small delta on the horizontal axis of each diagram represents the approximate position of the edge of the continental shelf (200 m) relative to the position of the stations. Certain similarities and differences in the distribution of volumes should be pointed out.

1. In the majority of sections (I, II, III, VI, VII, VIII) maximum zooplankton volumes for the shelf waters occurred at stations over the outer half of the shelf. The

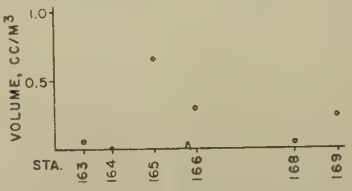

FIG. 4. Section I. The distribution of zooplankton volumes. May 1953.

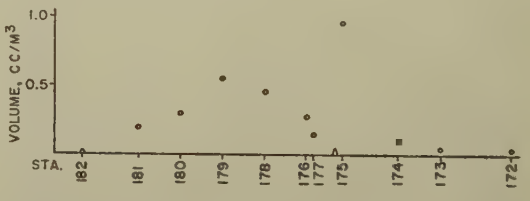

FIG. 5. Section II. The distribution of zooplankton volumes. May 1953.

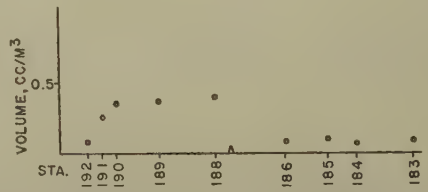

FIG. 6. Section III. The distribution of zooplankton volumes. June 1953.

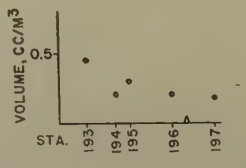

FIG. 7. Section IV. The distribution of zooplankton volumes. June 1953.

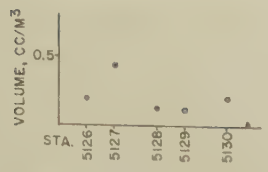

FIG. 8. Section V. The distribution of zooplankton volumes. January 1954.

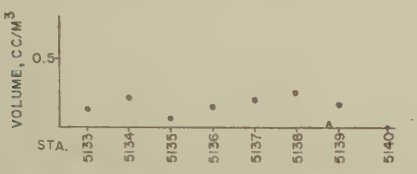

FIG. 9. Section VI. The distribution of zooplankton volumes. January 1954.

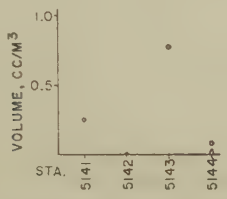

FIG. 10. Section VII. The distribution of zooplankton volumes. January 1954.

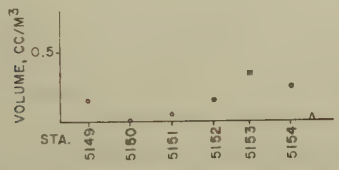

FIG. 11. Section VIII. The distribution of zooplankton volumes. January 1954.

maximum volume stations generally occurred near the 40-meter depth contour (Sections I, VI, VII, VIII), ranging from 32 meters in Section II to 54 meters in Section III. The greatest volume for all cruises, at Sta. 175, Section II, was due to a large accumulation of oceanic decapods.

2. Stations occupied near coastal areas on the spring cruises (Sections I, II, III) generally showed low volumes of zooplankton, and two sections which extended well beyond the edge of the shelf (Sections II, III) showed that the Florida Current also carries comparatively low volumes of plankton. The volume at Sta. 193 (Section IV) of the June cruise was unusually high for a coastal station. Most of the plankton volume at this station is attributed to copepods. The hydrographic data showed a penetration of the Florida Current into Raleigh Bay at the time this section was made. More than half of the numbers of individual copepods at Sta. 193 were most often associated in these samples with Florida Current water, attesting to the offshore origin of the water in this bay at the time of collection.

TABLE 2. *Summary of the average zooplankton volumes in cc/m³ for each section*

Section	Volume (cc/m³)
I	0.16
II	0.27
III	0.19
IV	0.26
V	0.23
VI	0.14
VII	0.27
VIII	0.16

3. In the January sections (V through VIII) relatively large zooplankton volumes occurred at stations occupied near the 20-meter depth contour (5126, 5134, 5141, 5149). Except at Sta. 5141 no reason to account for these increased volumes is apparent from examination of the hydrographic data. The water at Sta. 5141 was shown by Bumpus and Pierce (1955) to be of Virginian coastal water character, and this water had been transported southwestward around Cape Hatteras by a northeast storm (destroying the "temperature barrier") a few days previous to the collection of data from this area. The relatively large volume at Sta. 5141 and the peak at Sta. 5143 in Section VII was due to a single Virginian coastal calanoid, *Centropages typicus* Kröyer. The detailed distribution of this and other copepods will be reserved for a later paper. Table 2 summarizes the average zooplankton volume for each section.

COMPARISON WITH OTHER AREAS

Table 3 lists some areas in the eastern and western North Atlantic from which zooplankton volume data are available for months comparable to those of the present investigation. Volumes are given in cc/m³, and these values are averages of the volumes

TABLE 3. *A comparison of zooplankton volumes from selected areas in the North Atlantic Ocean*

Volumes measured by displacement or converted from settlement volumes. Values include total water column to a depth of 200 meters except where indicated.

Area	Authority	Zooplankton volumes in cc/m³		
		January	May	June
1. Cape Hatteras to Cape Fear	Present study	0.20	0.29	0.28
2. Cape Cod to Chesapeake Bay	Bigelow and Sears (1939)	0.40[1]	0.80	0.70
3. Georges Bank	Clarke and Bishop (1948)	0.20	1.10	1.50
4. Gulf of Maine	Bigelow and Sears (1939)	0.12	0.26[2]	0.26[2]
5. Nova Scotia to Newfoundland	Huntsman (1919)	—	0.23	0.13
6. Gulf of St. Lawrence	Huntsman (1919)	—	0.13[2]	0.13[2]
7. Iceland coast	Jespersen (1940)	—	0.45[3]	0.25[4]
8. Southern Norwegian Sea	Wiborg (1954)	0.01[5]	0.23[5]	0.34[5]
9. North coast of Norway (Eggum)	Wiborg (1954)	0.02	0.48	0.45
10. South coast of Norway (Ona)	Wiborg (1954)	0.01	0.06	0.08
11. Southwest North Sea	Savage (1931)	—	—	0.38[6]
12. English Channel	Russell (1927)	—	0.05	0.01
13. Skagerak	Con. Perm. I'Ex. Mer. (1904, 1905)	0.02[1]	0.05	—
14. Baltic Sea	Mielck and Künne (1935)	—	0.25[2]	0.25[2]

[1] February average.
[2] May–June average.
[3] Depth range 0–25 m.
[4] Depth range 0–50 m.
[5] Depth range 0–100 m.
[6] Depth range 0–65 m.

occurring at all stations occupied in a given area. Except where noted, the list of areas includes only coastal regions of 200 meters deep or less, and the values represent the volumes of zooplankton from the entire water column. Bigelow and Sears (1939) have previously compared the volumes of zooplankton from different areas in the North Atlantic, and to the data of these authors is added more recent observations from Georges Bank (Clarke and Bishop 1948), Iceland (Jespersen 1940), and Norwegian coastal and offshore waters (Wiborg 1954).

Some of the measurements of zooplankton volumes listed in Table 3 were made by "settlement" and some measurements by "displacement." Volumes measured by settlement usually give larger values than those measured by displacement, since in the former method the volume between the loosely packed organisms is also measured. Bigelow and Sears divided by a factor of 2 in converting settlement volumes to displacement volumes. Wiborg (1954), however, found in comparing the two methods using plankton collected from Norwegian coastal waters that settlement volumes gave values 1.6 to 6.6 times as large as values determined by displacement, averaging 4 times as large. In Table 3 all volumes originally reported by the settlement method have been converted to displacement volumes by dividing the original value by 4. Of the areas to be considered, only two were sampled using recording nets: the present study and Clarke and Bishop's (1948) investigation of the waters of Georges Bank. The zooplankton volumes of all other areas are based on estimates of the volume of water filtered by non-recording nets, given the diameter of the net and the length of the column through which the net was towed.

The most obvious features shown by a comparison of zooplankton volumes in North Atlantic coastal areas are: (1) the great variation in volumes from area to area during any one season, and (2) the marked increase in volumes in any one area from mid-winter to late spring. The highest average volume of zooplankton that has been reported in these areas occurred in Georges Bank water in June (1.5 cc/m³), and the second highest average value was from the same area in May (1.1 cc/m³). The maximum recorded zooplankton volume for a single haul in American waters is 6.9 cc/m³, which occurred over the outer half of the continental shelf off New Jersey in May of 1930 (Bigelow and Sears 1939).

Next to Georges Bank the coastal waters between Cape Cod and Chesapeake Bay appear to contain larger volumes of zooplankton during both winter and spring than any other temperate or boreal coastal area on either side of the Atlantic. The average zooplankton volume in May (0.8 cc/m³) in this area is almost twice that for

the next richest area, the Icelandic coast (0.45 cc/m³). The maximum volume reported by Jespersen for Icelandic waters was 1.45 cc/m³. From mid-winter to late spring zooplankton volumes are approximately doubled in the Cape Cod-Chesapeake Bay area, and volumes undergo a six- to seven-fold increase in Georges Bank waters over the same period.

Bigelow and Sears considered the shelf waters between Nova Scotia and Newfoundland to be richer in zooplankton than the Gulf of Maine, during May and June. Recalculation of Huntsman's (1919) data indicates, however, that the May-June zooplankton volumes for this shelf area (0.18 cc/m³) may be somewhat lower than volumes in the Gulf of Maine (0.26 cc/m³) for the same period. Bigelow and Sears also regarded the waters of the Gulf of St. Lawrence to be as rich as the Gulf of Maine for the May-June period. Recalculation of Huntsman's data indicates that zooplankton volumes in the Gulf of St. Lawrence for these months (0.13 cc/m³) are about one-half of the volumes in Gulf of Maine waters.

North European waters in general average poorer in zooplankton than the richest areas in the western North Atlantic. The maximum average zooplankton volume reported from North European waters is 0.63 cc/m³ from the northern coast of Norway during July (Wiborg 1954), although single hauls in this area occasionally yielded zooplankton volumes exceeding 5.0 cc/m³. Volumes off the southern coast of Norway are very low (maximum: 0.08 cc/m³ in June). Savage (1931) reported that zooplankton volumes in the North Sea in June averaged about 1.15 cc/m³ as measured by settlement, and Bigelow and Sears converted this volume to 0.57 cc/m³ by displacement. Savage, however, states that settlement volumes in the North Sea samples were about three times the displacement volumes, and hence the corrected value for zooplankton volumes should be 0.38 cc/m³ for that area. Zooplankton in the southern Norwegian Sea (Weather Station M) reaches a maximum volume in June (0.34 cc/m³) and a minimum during December, January, and February (0.01 cc/m³).

The remaining areas listed in Table 3 (Skagerak, Baltic Sea, English Channel) are considerably poorer in volumes of zooplankton, although volumes in the Baltic during May and June are similar to those in the Gulf of Maine and the southern Norwegian Sea. Average zooplankton volumes in European waters in mid-winter are extraordinarily low compared to volumes in western Atlantic waters.

Zooplankton volumes from Cape Hatteras to Cape Fear (the present study) are similar to volumes in the Gulf of Maine and the southern Norwegian Sea during the May-June period, and about $\frac{3}{5}$ as great as values reported from the rich northern Norwegian coastal waters. Winter volumes in the present area are similar to those for Georges Bank during January, about $1\frac{1}{2}$ times the volumes reported from the Gulf of Maine, and about one-half the volumes of the rich Cape Cod-Chesapeake Bay area. The mid-winter to late spring differences in any one area are generally quite large in the areas listed in Table 3. Winter volumes in the Cape Cod-Chesapeake Bay area are about 53% of the May-June average, and in Georges Bank waters the January average is about 15% of the May-June volumes. Extreme differences occur off the north coast of Norway at Eggum where the January volumes are only about 4% of the May-June average. By contrast, there appears to be no pronounced mid-winter minimum of zooplankton volumes in the present area, where the January 1954 volumes were maintained at about 70% of the preceding May-June average.

DISCUSSION

The relatively slight difference in late spring and mid-winter zooplankton volumes in the present area appears in marked contrast to other coastal areas of the North Atlantic which show wide differences in zooplankton volumes during these two seasons. The local hydrographic conditions appear to play an important role in accounting for the relatively constant seasonal volumes. The Florida Current occasionally penetrates shoreward over the

North Carolina shelf and carries into coastal waters many oceanic species. The presence of Florida Current water over the shelf could not in itself increase by any great amount the volume of zooplankton over the shelf, since the waters of the Current have been shown to carry very meager quantities of plankton compared to that in coastal waters.

There is some evidence to indicate that reproduction may occur among the oceanic species, however, after their arrival in coastal waters from the Florida Current. In the samples taken in coastal water in Onslow and Long Bays during January, there invariably occurred copepodid stages of copepods usually considered as "oceanic" (*Euchaeta marina*, *Temora stylifera*, *Nannocalanus minor*, for example) together with adult males and females carrying attached spermatophores. In the case of one oceanic species, *Euchaeta marina*, females bearing egg cases were common in the samples. The mesh of the nets employed for collection was too coarse to retain nauplii or the younger copepodid stages of the smaller species of copepods, but the presence of the later immature stages and reproducing adults indicates that reproduction of oceanic species in these waters during January provides a potential for adding to the winter calanoid population which, in part, is responsible for maintaining relatively high winter volumes.

The proximity of the Florida Current to the North Carolina shelf may have important consequences other than the fact that this Current is a primary source of offshore species. Winter water temperatures in the present area are considerably higher than temperatures of adjacent waters a few miles northward. Directly north of Cape Hatteras winter surface temperatures are generally 8 to 10° over the 200-meter depth contour, while during the present January cruise temperatures over the edge of the shelf in the two more southern sections were above 20°. Shoreward penetrations of the Florida Current carry warm waters well inshore of the edge of the shelf so that in Onslow and Long Bays the 15° isotherm is never more than 30 miles offshore (the mid-shelf region) during the winter months for which data are available. Barring occasional southwestward movements of water from north of Cape Hatteras Bumpus (1955) has shown that the 15° isotherm in Raleigh Bay occurs in January and February in very shallow water only a few miles seaward to the offshore bars.

Low temperature *per se* probably is not a direct limiting factor to the existence of zooplankton in coastal waters during the winter months; temperature acts indirectly, together with reduced solar radiation and turbulence during the winter months, by reducing the production of phytoplankton which forms the basic food supply of herbivorous species of the zooplankton (Riley 1942). The reduction in the standing crop of phytoplankton in temperate coastal areas from spring to winter is one of the more important factors tending to reduce the size of the zooplankton population during the colder months.

Fish (1954) and Riley (1957) have reported the results of zooplankton and phytoplankton collections made over a two-year period at weather station "E" in the north Sargasso Sea. Although this is a mid-oceanic position, Sta. E is located at about the same latitude as Cape Hatteras and the solar radiation is, therefore, about the same. The volumes of zooplankton showed a spring increase (peaks in April of 1950 and in May of 1951) with lower values during the late summer and autumn months. Peaks in phytoplankton volumes generally preceded the zooplankton maxima by about a month. Riley showed that the summer and autumn minimum in volumes was due to a persistence of the thermocline through November, preventing the replenishment of nutrients in the upper layers. These authors also show a winter increase in plankton volumes over the autumn minimum, and Riley attributes this to the high light intensity at the latitude of Sta. E which reduced the requirement of a highly stable water column.

Since winter solar radiation at the latitude of the Carolinas is sufficient to maintain at least a moderate rate of productivity of phytoplankton, and hence zooplankton, it

would seem that a seasonal variation in volumes may depend in large part on the available supply of nutrients. The North Carolina shelf is a relatively shallow area, averaging 40 meters or less in depth, and since the hydrographic data for January show these waters to be homogeneously mixed from surface to bottom, there appears no reason why the phytoplankton in these waters should not have a sufficient nutrient supply available during the winter months, hence accounting for the relatively high winter zooplankton volumes reported here. Whether the thermocline persists through late autumn off the Carolinas thereby reducing plankton volumes during this season (as occurs at Sta. E) cannot be decided as yet for the present area.

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Ecological Studies on the Planktonic Protozoa of a Small Artificial Pond[1]

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ABSTRACT

A one-year survey was conducted on a small artificial pond to determine environmental relationships of planktonic protozoa to physical and inorganic chemical factors.

Collections were made at 9:00 A. M., Eastern Standard Time, every other day. Planktonic samples were taken from the surface and bottom, and sunlight intensity and temperature were measured. Chemical analyses included alkalinity, carbon dioxide, chloride, dissolved oxygen, nitrate, nitrite, ammonia, phosphate, pH, hardness, and turbidity. Bacteria counts were made microscopically, and protozoa were handled by the Sedgwick-Rafter method.

The order of appearance of phytoplankton may have been influenced by the abundance of growth factors coupled with varying amounts of dissolved substances: *Cryptomonas* appeared in autumn and early summer; chrysomonads and *Chlamydomonas* in winter; *Glenodinium*, *Pyramimonas*, and diatoms in spring; and euglenoids in summer.

The amount of phosphate seemed to influence which phytoplankters appeared: chrysomonads were not found when the water contained more than 0.02 ppm of phosphate, whereas cryptomonads and diatoms increased at this concentration, and euglenoids were abundant at several times this concentration. Within each phosphate range other factors, such as nitrogen sources and growth factors, exercised more immediate effects. Supply of organic nutrients appeared to influence euglenoid numbers.

Increases in sunlight and temperature were favorable for diatoms but unfavorable for *Glenodinium cinctum* and *Trachelomonas hispida*.

The nature and amount of food appeared to be the controlling factor for ciliates.

Many plankters live perilously close to minimum survival conditions, and slight environmental changes can promote rapid growth, reduction, or subsidence of a population. This survey shows that detailed studies are necessary to ascertain ecological relationships of microorganisms, and that protozoan habitats must be defined more precisely.

INTRODUCTION

Weekly collections from a small body of water, such as a pond, provide a general picture of its ecology. However, since sudden changes can and do occur in ponds within a week, more frequent sampling may yield critical information on the interrelations of organisms and environmental factors.

This paper reports a one-year survey conducted on a small artificial pond to determine environmental relationships of planktonic protozoa to certain physical and inorganic chemical factors known to be fundamental in protozoan metabolism. Collections were made every other day over a period of one year, and were supplemented by data collected in other years on the same pond.

Many people have expressed interest in this project. In particular, the writer thanks his wife for technical assistance, and Dr. D. H. Wenrich and Dr. Luigi Provasoli for guidance and encouragement.

GENERAL DESCRIPTION OF POND

This survey was conducted on the small artificial pond in the Botany Gardens at the University of Pennsylvania. The oval pond measures 37 by 18 meters, has a surface area of about 450 square meters, and a volume of about 240 cubic meters. The greatest depth (at the center) is just over a meter, while the average depth is between 40 and 60 centimeters. Rainfall cannot maintain the water level, and consequently

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the pond is fed from time to time from the Philadelphia water supply.

From spring to autumn iris and other plants adorned the borders, and a luxuriant growth of pond lilies covered the surface. A moderate growth of *Chara* constituted the principal submerged vegetation. The bottom was covered by a large amount of decaying vegetation, primarily leaves from nearby trees.

The sparse non-flagellated phytoplankton included *Oscillatoria*, *Spirogyra*, *Scenedesmus*, and diatoms. The latter exhibited a population surge in late spring.

Protozoa, predominantly green flagellates, constituted the bulk of the plankton. Rotifers appeared in large numbers in May and remained in moderate numbers throughout summer and autumn. Gastrotrichs, nematodes, oligochaetes, and copepods were few and infrequent. About 200 goldfish, some frogs, and turtles, constituted the vertebrate fauna.

This pond had previously been surveyed by Wang (1928), who found a moderate flagellate and rich ciliate population. The pond was partially cleared in 1933, but since then it has filled in considerably with organic debris, and the flagellate population has increased while the ciliate population has decreased.

METHODS

Collections

Collections were made at 9:00 A.M., Eastern Standard Time, every other day from October 9, 1955, to October 9, 1956, (184 collections) at a rock-bordered projection on the northwest side of the pond.

Surface collections were made by lowering a pint jar just below the water surface. Collections of plankton just above the bottom were made by an instrument modified from Noland's (1925) apparatus. A three-foot brass rod was attached by a universal joint to a perforated square plastic plate. An eighth-inch hollow plastic tube, closed at the lower end, was attached to the rod in a parallel position so that the lower end of the tube was one inch above the bottom surface of the plate. Lateral holes in the tube allowed water to enter. The open end of the tube was connected by rubber tubing through a two-hole rubber stopper that fitted into a pint jar. A tube passed through the other hole into the collector's mouth so that suction could be applied to draw pond water into the jar.

A Weston illumination meter, model 756, specially built and calibrated by the manufacturer for a ten-foot waterproof cable between the (waterproofed) photoelectric cell and meter, was used to measure sunlight intensity. The photoelectric cell was attached to the plate of the collection instrument. Readings were made at the surface of the pond, beneath the ice, and at the bottom.

Water samples for chemical analysis were collected in jars, and those for dissolved oxygen determination in glass-stoppered bottles. Sunlight intensity, air and water temperatures, pond level, and notes on the weather and pond were recorded at the time of collection.

Biological analysis

Jars containing samples for biological analysis were shaken. Ten cc were then removed for bacteriological counts, which were performed microscopically, using a Levy counting chamber (hemocytometer).

The plankton in the remaining water was concentrated by filtration in Sedgwick-Rafter funnels (Whipple 1927). All filtration was carried out in a cold room (kept at 14°C), and concentrates were stored there until examined. For samples taken in winter, ice was placed in jackets around the funnels, and organisms were stored in a refrigerator prior to examination. These precautions delayed degeneration due to higher temperatures.

Organisms were examined in a Sedgwick-Rafter counting cell. The concentrate was introduced into the cell slowly by a wide-mouthed pipette. Rotifers and ciliates, because of their sensitivity to heat under the microscope, were counted first, flagellates afterward. The cell was first examined under a dissecting microscope and all the large forms counted. Under the compound microscope, between 70 and 100 fields in the cell were counted. Two, and sometimes

three and four, cells were examined and the results averaged. Since flagellates were the most numerous organisms, 20 fields within the cell sufficed to give a reliable count, but when the numbers fell to less than five organisms per field, 40 to 60 fields were counted.

Every two weeks plankton collections were made (at surface and bottom) at four other stations around the edge of the pond to see if the alternate-day samples were representative of the entire pond. Occasionally chemical tests were made at these other stations for the same purpose.

Chemical analyses

Chemical analyses were performed within two hours after collection. The following analyses (with methods in parentheses) were performed according to American Public Health Association (1955) procedures: alkalinity (methyl-orange titration), chloride (Mohr), dissolved oxygen (Winkler), nitrate (phenoldisulfonic acid), nitrite (diazotization), ammonia (direct Nesslerization), phosphate (stannous chloride), and pH (Beckman H-2 pH meter). Total and calcium hardnesses were determined by the borate buffer procedure of the ethylenediaminetetraacetic acid (EDTA) method (A. P. H. A., 1955, pp. 116–118), using reagents manufactured by the W. H. and L. D. Betz Company, Philadelphia, Pa. The indicator and buffer reagents employed were in powdered form, and hence not subject to deterioration, otherwise the method was the same as A. P. H. A. Magnesium hardness was found by subtracting calcium from total.

Carbon dioxide was found from a graph (Betz 1955) showing the relationship of carbon dioxide, alkalinity, and pH.

Turbidity measurements were read on a Klett-Summerson colorimeter (using a blue filter) and compared to a diatomaceous earth solution which had been standardized in a Jackson candle turbidimeter.

Presentation of data

Graphs were constructed for all physicochemical factors measured and for dominant organisms and groups of organisms. The most significant graphs are presented in this paper. Fifteen-point moving averages were computed for data on sunlight intensity, temperature, daily length of sunlight, and for major groups of organisms. Graphs are compared with each other and with those in the literature to seek relationships between environmental factors and organisms.

Three other sets of observations on this pond are utilized in this paper: (1) Wang's survey (1928); (2) the writer's six-month survey, October 15, 1953 to April 15, 1954 (unpublished master's thesis); and (3) author's series of weekly observations, October 9, 1956 to February 2, 1957.

In the presentation of data, results are given, followed by discussion, for each physical and chemical factor and group of organisms.

RESULTS

Validity of counting technique

The precautions in using the Sedgwick-Rafter counting cell, outlined earlier (under biological analysis), were based on the author's experience and suggestions by Serfling (1949), who showed that Sedgwick-Rafter counts tend to deviate from a Poisson distribution when numbers are low. Because most counting of organisms was done on living material, the χ^2 index of dispersion was calculated for some of the data to ascertain whether the distribution of organisms in the counting cell differed from the expected Poisson distribution. Examples of these calculations are presented in Table 1.

The distribution of single-celled organisms and *Dinobryon* agreed well with a Poisson distribution. To ascertain how well counts agreed for each organism, the χ^2 and degrees of freedom were totaled from groups of ten and the normal deviate calculated by the formula presented by Yule and Kendall (1950, p. 665). Normal deviates for *Chlamydomonas incerta*, *Leptocinclis fusiformis*, and *Dinobryon sertularia* were 1.89, 1.70, and 1.31, respectively, which are not statistically significant.

Synura uvella and *Gonium pectorale*, however, often deviated significantly from a Poisson distribution, because the colonies

TABLE 1. *Comparison of counts of selected phytoplankters with a Poisson distribution by the χ^2 method*

	Chlamydomonas 3/11/56	*Dinobryon* 1/29/56	*Leptocinclis*		*Synura uvella*		*Gonium pectorale*	
			7/29/56	8/12/56	12/12/55	1/11/55	3/21/56	3/29/56
Field counts per Sedgwick-Rafter cell	7	18	2	5	3	11	18	6
	11	12	1	3	17	6	28	6
	6	30	0	7	9	9	13	4
	4	20	1	9	11	2	7	11
	10	11	1	8	16	9	12	4
	7	15	1	3	14	10	10	6
	9	16	1	5	18	8	6	7
	2	24	1	2	16	8	10	3
	6	21	2	6	15	10	5	3
	8	13	0	6	18	7	6	13
	6	13	1	5	17	10	4	4
	9	10	0	3	9	12	4	2
	6	13	2	6	24	9	13	2
	8	19	1	3	16	9	6	3
	8	18	2	6	17	17	18	4
	5	22	0	5	21	14	7	4
	11	19	0	2	11	19	5	8
	7	19	0	3	28	8	7	3
	13	16	0	3	14	4	10	8
	15	19	5	4	30	13	16	3
Total	158	348	21	94	324	195	205	104
χ^2	22	19	27	19	29	30	64	33
Deg. of freedom	19	19	19	19	19	19	19	19
Prob.*	.20–.30	.30–.50	.10–.20	.30–.50	.05–.10	.02–.05	.01	.02–.05

* A probability less than .05 is generally considered as deviating statistically from a Poisson distribution.

tended to aggregate in the counting chamber. The normal deviates for groups of ten for *Synura* and *Gonium* were 4.33 and 5.31, respectively, which are considerably greater than the 1.96 limit for statistical reliability. Hence, such forms are better counted in the fixed condition.

The reliability of the counting procedures employed in this survey satisfy the conditions set forth in Kutkuhn's (1958, pp. 77, 80, 81) analysis.

Many of the population curves showed extreme fluctuations. To distinguish between counting errors and real changes, 95% confidence intervals were calculated for several organisms by doubling the square root of each count and plotting this value as the error for each count. An example is presented in Figure 1 for *Chlamydomonas incerta*. These confidence intervals were typical of those of other species and give some idea of the reliability of the determinations.

Fortnightly samples

To determine whether samples from a single station adequately reflected the pond's population, fortnightly surveys were made at four other points around the pond's periphery. Table 2 summarizes the data for dominant organisms. Marked fluctuations sometimes occurred at the north end, more frequently at the south end. These areas are very shallow and support dense growths of *Spirogyra* in winter. In general, sampling at one station in this pond reflects the general biota most of the time; although the difference between the numbers at any two sampling stations may be greater than the limits of the counting errors (95% confidence intervals).

Sampling of the chemical environment at various places in the pond showed greater homogeneity than the biotic.

Daily periodicity

Chemical changes in a pond can be great within a twenty-four hour period. Meas-

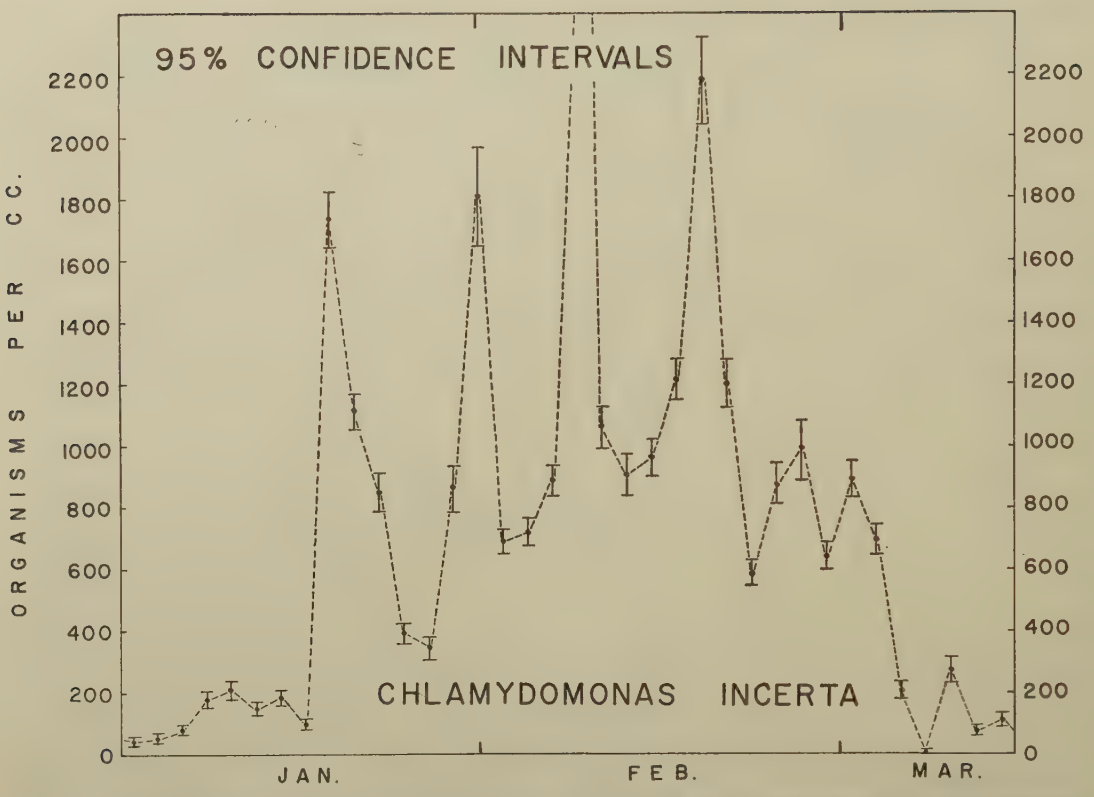

FIG. 1. Ninety-five per cent confidence intervals calculated for a population of *Chlamydomonas incerta*, to show the reliability of determinations made by the Sedgwick-Rafter counting method employed in this survey.

urement of daily periodicity lay outside the scope of this project, but a 48-hour survey was carried out April 22–23, 1955, to see how representative of maximal values the 9:00 A. M. collections were. The maximum values for temperature, pH, and dissolved oxygen occurred in midafternoon and the minimum in early morning. For ammonia the maximum occurred in early morning and the minimum near noon, nitrate increased slightly at night, and phosphate and chloride showed slight and irregular changes. Nitrite was barely detectable. The data for temperature, pH, dissolved oxygen, and ammonia are shown in Figure 2.

Measurements made at 9:00 A. M. for temperature, pH, and dissolved oxygen are low because these factors reach their maximum later in the day. Ammonia readings are also low, but for the opposite reason. The daily values are comparable in the sense that they were taken at 9:00 A. M. each day. However, due to changing day lengths, the values are not strictly related.

The physico-chemical environment

Generally speaking, the physical and chemical factors followed one of two trends. The temperature curve showed a trough in winter and a peak in summer, the latter period characterized by high quantities of ammonia, phosphate, and carbon dioxide. Dissolved oxygen, nitrate, and pH followed a different trend, being highest in winter and spring.

The difference between the temperature of the water and that of the air ranged from two to five degrees C. The 9:00 A. M. air temperature averaged three to four degrees above the daily mean (computed from U. S. Weather Bureau records), so that the data on water temperature shown in Figure 3 probably average a degree or two above the daily mean.

TABLE 2. *Surface counts (organisms per cc) of dominant organisms in fortnightly surveys*

Dominant organisms	Date	Stations around periphery of pond				
		North	East	South	West	Northwest*
Cryptomonas compressa	Nov. 23	124	85	189	70	164
Dinobryon sertularia	Feb. 1	86	481	317	325	400
Chlamydomonas incerta	Feb. 1	909	1412	173	716	870
Chlamydomonas incerta	Feb. 15	1350	456	2408	1176	964
Chlamydomonas incerta	Feb. 29	524	421	742	564	645
Chlamydomonas incerta	Mar. 14	299	198	60	81	115
Pyramimonas minima	Apr. 11	436	436	206	462	483
Chlamydomonas globosa	Apr. 11	669	777	535	782	459
Diatoms	Apr. 25	739	652	815	860	738
Diatoms	May 9	1784	1121	1487	889	1146
Glenodinium cinctum	Apr. 25	512	2104	25	893	836
Glenodinium cinctum	May 9	37	81	98	19	61
Trachelomonas volvocina	Aug. 1	1323	451	595	277	349
Trachelomonas volvocina	Aug. 13	381	405	197	248	252
Leptocinclis fusiformis	Aug. 13	254	443	532	239	416
Leptocinclis fusiformis	Aug. 29	1068	1276	291	300	1266

* Major collection station for the survey.

A summary of sunlight intensity at 9:00 A. M. is presented in Figure 3. The "maximum possible" curve A was drawn connecting points of those days of greatest sunlight intensity. On some cloudy days which yielded low intensity readings, the sun would come out later in the day, and sometimes the converse would happen on bright clear days (*i.e.*, clouding over). These two deviations tended to cancel each other, since the curve B of average readings parallels that of per cent possible hours of sunlight, computed from U. S. Weather Bureau records. About 1000 foot-candles should be added to winter readings of curve A and about 2500 foot-candles to summer readings to obtain the maximum sunlight intensity, which occurs between noon and 3:00 P. M.

pH ranged from 5.9 to 8.8. Fluctuations in the pH graph often coincided with fluctuations of dissolved oxygen, and the two factors followed the same trend throughout the year. From December to May the water was supersaturated with oxygen. The large content in winter was due in part to the ice sheet, which prevented diffusion into the air. The maintenance of supersaturation after January was due to photosynthetic activities of the phytoplankton: peaks in the dissolved oxygen graph coincided with those of the plankton, and the drop in late March paralleled a drop in the plankton population.

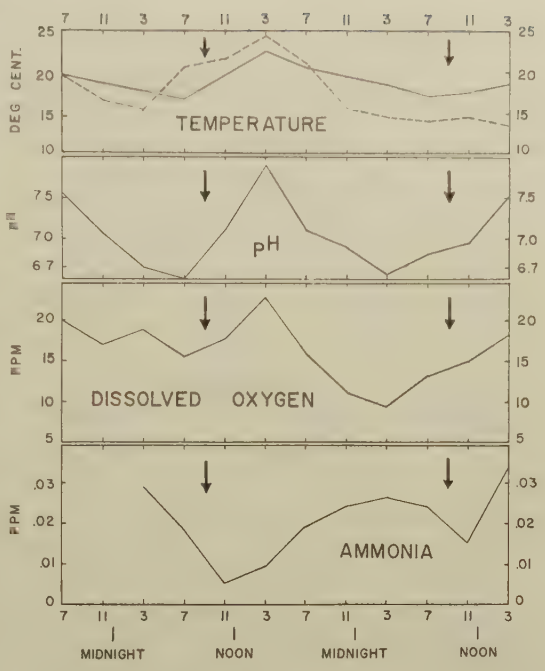

FIG. 2. Forty-eight hour survey, carried out April 22–23, 1955. Arrows indicate 9:00 A. M., the collection time used during the survey.

Organic nitrogen was not determined. Ammonia, nitrite, and nitrate nitrogen are reported as ppm of nitrogen. Study of Figure 4 shows that almost all of the inorganic nitrogen was represented by ammonia. Water in the city supply, which is piped into this pond from time to time, contained twice as much nitrate as was found in the pond, but was practically devoid of nitrite or ammonia. This amount of nitrate (0.02 to 0.03 ppm of nitrogen) is inadequate to supply more than a small fraction of the ammonia present in the pond. Increases in ammonia production must be due to the

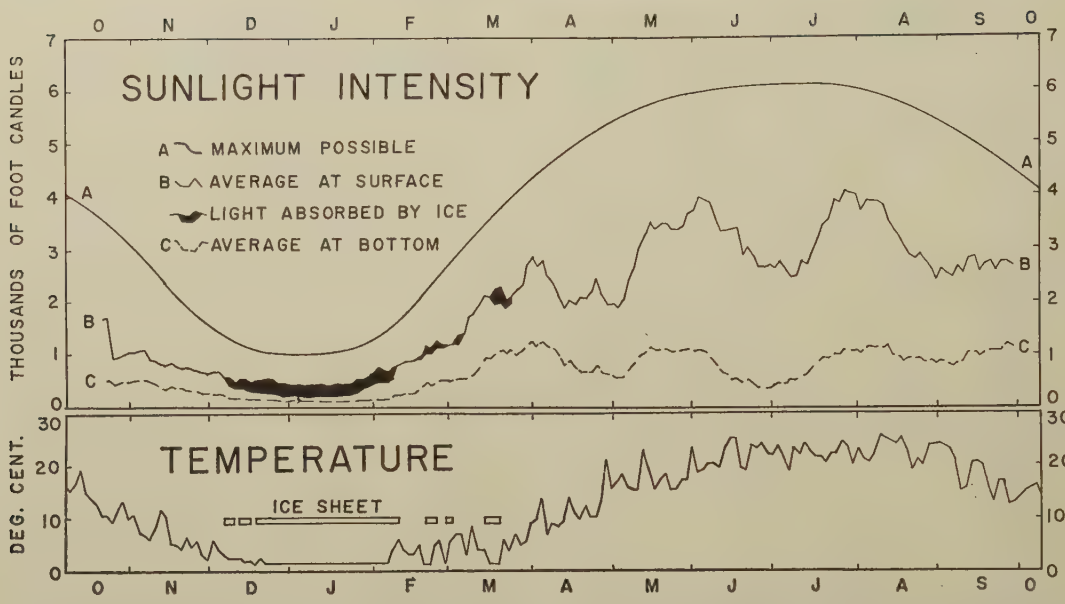

FIG. 3. Physical factors. Readings in this and all subsequent graphs were taken at 9:00 A. M. About 1000 foot-candles should be added to winter readings and about 2500 to summer readings to obtain the maximum sunlight intensity for the day.

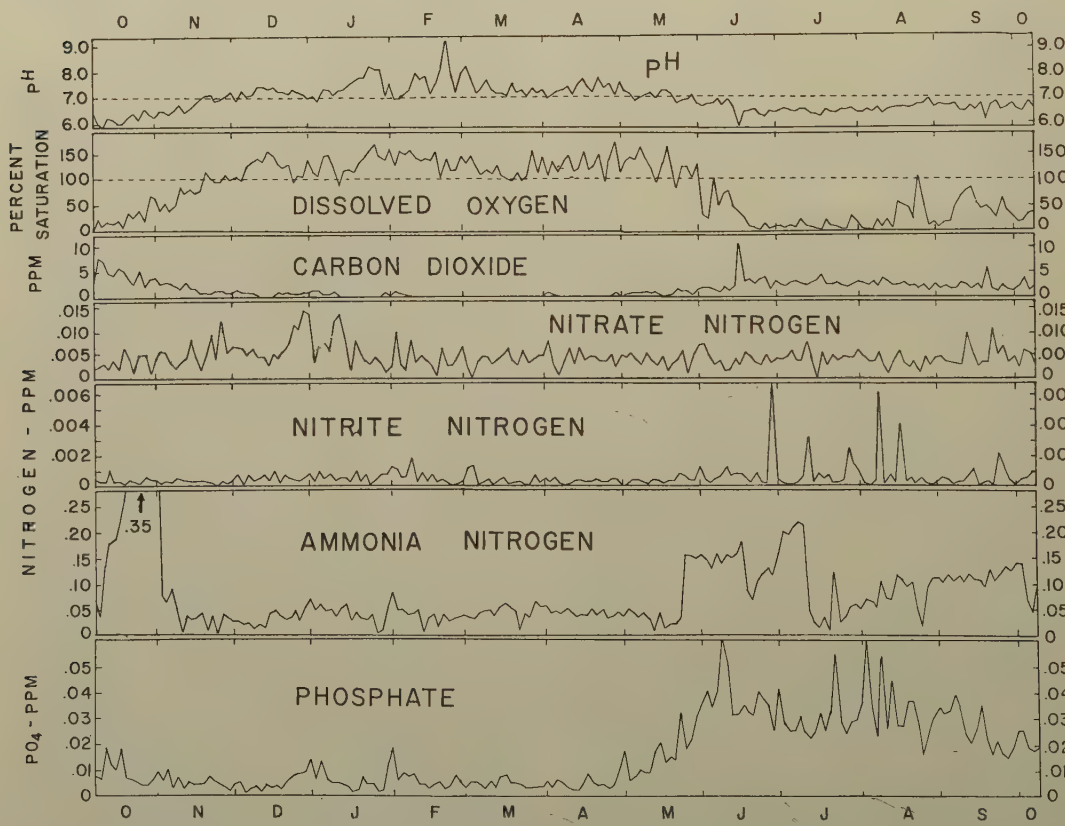

FIG. 4. Distribution of inorganic chemical factors. A seasonal cycle can be traced in all curves except nitrite, which is characterized only by extreme fluctuations in the summer.

conversion of organic compounds into ammonia.

Nitrite was between 0.0005 and 0.001 ppm nitrogen throughout most of the survey, but in summer exhibited sudden surges which subsided as quickly as they appeared (Fig. 4.) These bursts occurred when the oxygen content was almost exhausted and usually coincided with increases in ammonia. These data agree with Mortimer's (1941) findings that the main source of nitrite in freshwater bodies is through reduction of nitrate, not oxidation of ammonia. This is particularly true when oxygen falls below two ppm.

Nitrate was most abundant in December and early January.

Phosphate, reported here as the radical, averaged less than 0.01 ppm from autumn to spring, but increased threefold during May and exhibited a number of sharp fluctuations through the summer (Fig. 4). These fluctuations coincided with fluctuations in turbidity, and to some degree with fluctuations in ammonia.

Hardness measures calcium and magnesium ions, and also gives an approximate indication of bicarbonate carbon dioxide. The calcium hardness increased to 110 ppm in winter but declined rapidly in February to 50 ppm coincident with the winter increase in phytomonad population, remained low during spring (a period of phytoplankton increase), and rose again in July. The period of low values may reflect photosynthetic activity, because chlorophyll-bearing organisms remove free carbon dioxide from the water, thereby upsetting the bicarbonate equilibrium. Under these conditions, the bicarbonate decomposes to form water, carbon dioxide, and carbonate, which precipitates; hence the bicarbonate content of the water is diminished (Ruttner 1953).

The magnesium hardness remained high (80 ppm) during the winter phytomonad surge and declined (to 40 ppm) in late May, after the spring phytoplankton surge declined (Fig. 8). Comparison with other graphs revealed no relationships, except a period of great fluctuation in April, during the spring phytomonad increase.

Chloride in this pond showed the same trend as chloride in the city water supply, which generally followed the trend of sunlight and temperature. Daily fluctuations were slight, and chloride ranged between two ppm in winter and 10 ppm in summer. The greater concentration in summer reflects more frequent filling of the pond from the city supply.

Turbidity averaged 30 ppm in the autumn and winter, and ranged between 50 and 130 ppm in spring and summer. The large phytoplankton population in late winter and spring increased turbidity, as did heavy rainfalls, but in general, fluctuations in turbidity coincided with or just preceded those in phosphate, and to a lesser extent, those of bacteria. Turbidity is caused by the presence of plankton (including bacteria) and detritus. The twofold increase in summer, despite low plankton numbers (compared with spring), indicated a large amount of detritus in the water. The accompanying phosphate increases suggest decomposition processes which produced the detritus.

Bacteria

Bacteria (Fig. 5) were numerous in October, during the period of high ammonia concentration, and in February, following the January population of *Dinobryon sertularia*. The low numbers in May and the summer may reflect predation by rotifers and ciliates, since most of the species observed feed on bacteria and detritus. These observations agree with Hammann's (1952) deduction that the numbers of bacteria do not necessarily parallel the degree of organic pollution.

Phytoplankton

The principal groups of the plankton are discussed in the order they appeared in the survey. Both surface and bottom planktonic samples were collected. For most organisms, the difference between the two populations was little greater than the counting errors (95% confidence interval). However three species—*Synura uvella*, *Dinobryon sertularia*, and *Gonium pectorale*—showed significant differences between surface and bottom populations. These differences are noted below.

Cryptomonads were represented by *Cryptomonas compressa* and *C. erosa*. They appeared at the start and finish of periods

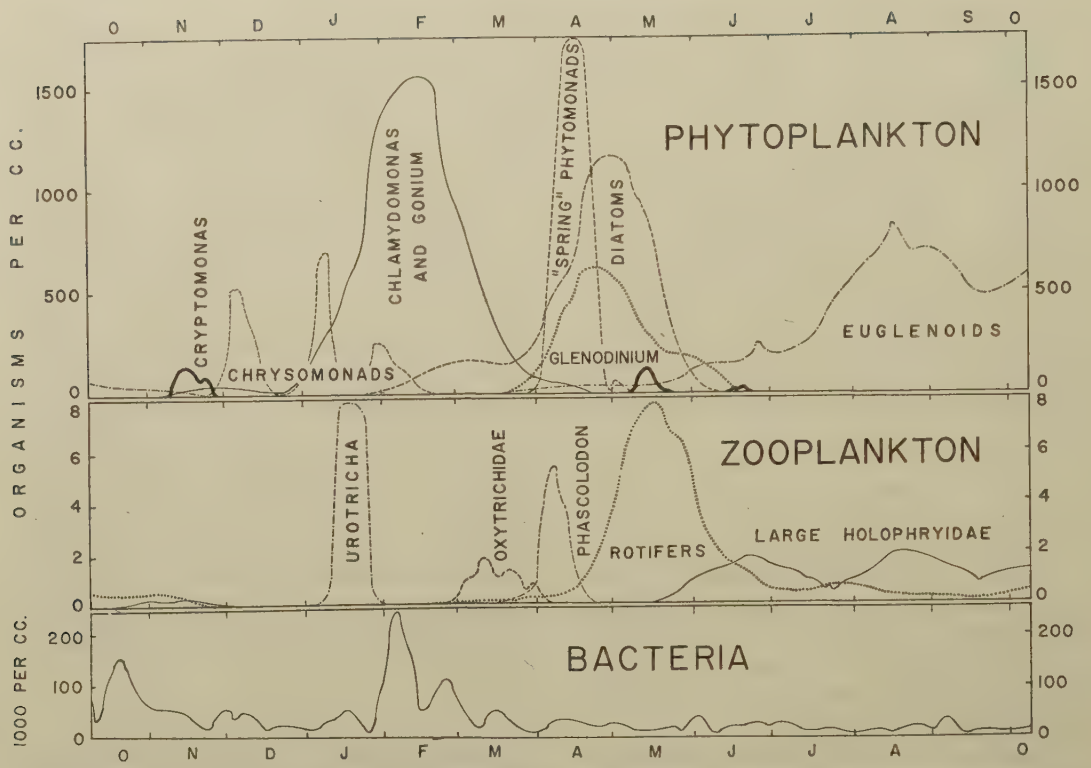

FIG. 5. The succession of dominant organisms. The curves are smoothed by fifteen-point moving averages.

of decomposition: in November, middle of May, and middle of June (Figs. 5 and 9). In the author's winter survey of 1953–54, the genus appeared in November. These appearances suggest that environments with a moderate abundance of organic nutrients but low ammonia concentration influence the organisms' distribution. This agrees with other workers' (*e.g.*, Pascher 1926, Lund 1942) observations in nature, and Provasoli and Pintner's (1953) findings that *Cryptomonas* requires growth factors but exhibits a low osmotic tolerance.

Three species of chrysomonads furnished the dominant population in early winter: *Sphaleromantis ochracea*, *Synura uvella*, and *Dinobryon sertularia*. *Sphaleromantis* was found in approximately equal numbers at both surface and bottom; it exhibited two population surges which coincided with those of *Synura*. *Synura uvella* was several times more numerous at the surface than at the bottom, and tended to appear during declines in ammonia production (Fig. 6). As the second *Synura* and *Sphaleromantis* surge declined, a population of predaceous ciliates belonging to the genus *Urotricha* suddenly appeared, which may have prevented these chrysomonads from reappearing, since the ciliates' bodies were full of chrysomonad inclusions.

Dinobryon was twice as numerous at the bottom as at the surface and showed an inverse relationship with bacteria (Fig. 6). The sudden decline of the species coincided with a sudden rise in phosphate to 0.02 ppm.

The occurrence of *Synura* at the surface is not surprising, since the species is very motile and is an obligate phototroph. For *Dinobryon*, on the other hand, motility is decreased by a lorica. The concentration of this flagellate at the bottom under poor sunlight conditions (averaging less than 50 foot-candles) and its inverse relationship with bacteria suggest heterotrophic abilities, already suspected by some workers (*e.g.*, Provasoli, personal communication). The

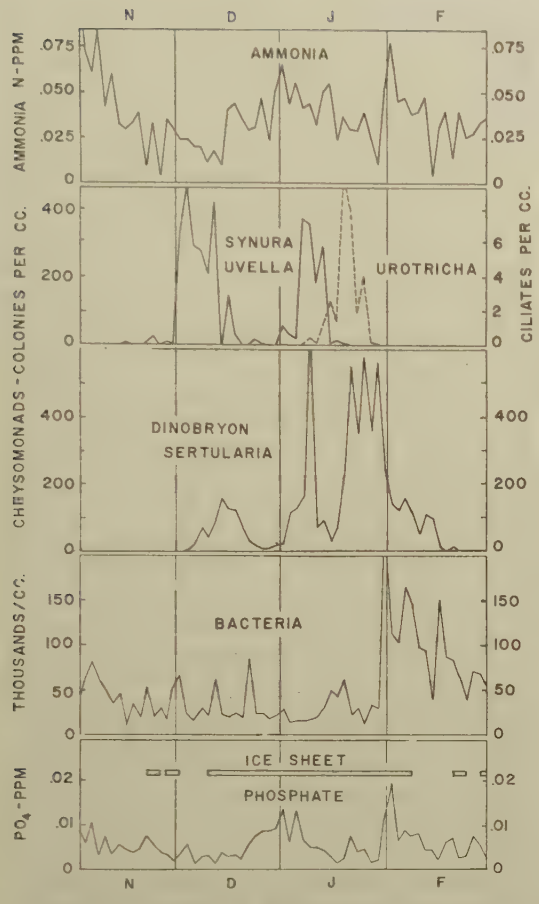

FIG. 6. Distribution of dominant chrysomonads and related factors. *Synura* disappeared after predation by *Urotricha*. The disappearance of *Dinobryon* after the rise in PO_4 agrees with similar observations by Rodhe (1948).

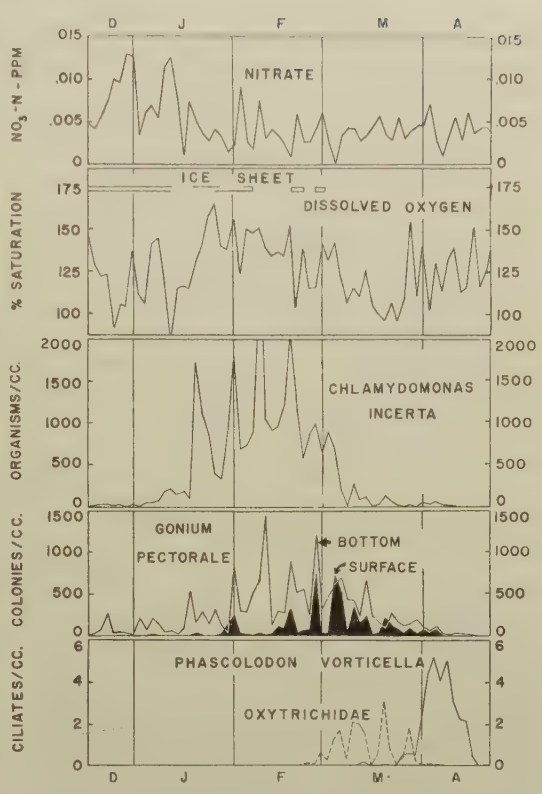

FIG. 7. Occurrence of winter phytomonads and related factors. The lag period between the decline of the flagellates and the appearance of their ciliate predators agrees with Proctor's (1957) antibiosis observations on *Chlamydomonas*.

disappearance of *Dinobryon* at 0.02 ppm of phosphate is supported by Rodhe's (1948) observation that concentrations above 0.015 ppm of phosphate are lethal to this organism. Provasoli and Pintner (1953) have reported that *Synura* exhibits low phosphate tolerances.

The phytomonads were represented by two distinct appearances, one in winter dominated by *Chlamydomonas incerta* and *Gonium pectorale*, and the other in the spring consisting of *Pyramimonas minima* and *Chlamydomonas globosa*. Generally speaking the winter species fluctuated with dissolved oxygen but often inversely with nitrate (Fig. 7). *Chlamydomonas incerta* was present in about equal numbers at both surface and bottom, but *Gonium pectorale* was more abundant near the bottom.

The spring phytomonads—*Pyramimonas minima* and *Chlamydomonas globosa*—appeared in April when the water temperature rose 8°C (Fig. 8). Competing populations of diatoms and *Glenodinium cinctum* accelerated as the phytomonads declined in May. The large numbers of flagellates in April and May indicated intense competition, and the decline of light intensity in late April may have favored the yellow-brown organisms over the phytomonads.

The dinoflagellates were represented almost exclusively by the species *Glenodinium cinctum*. This flagellate occurred through most of the survey except June and July, with a population surge in April and early May, when the organism was succeeded by a large population of diatoms. Apart from this survey, three other observations on

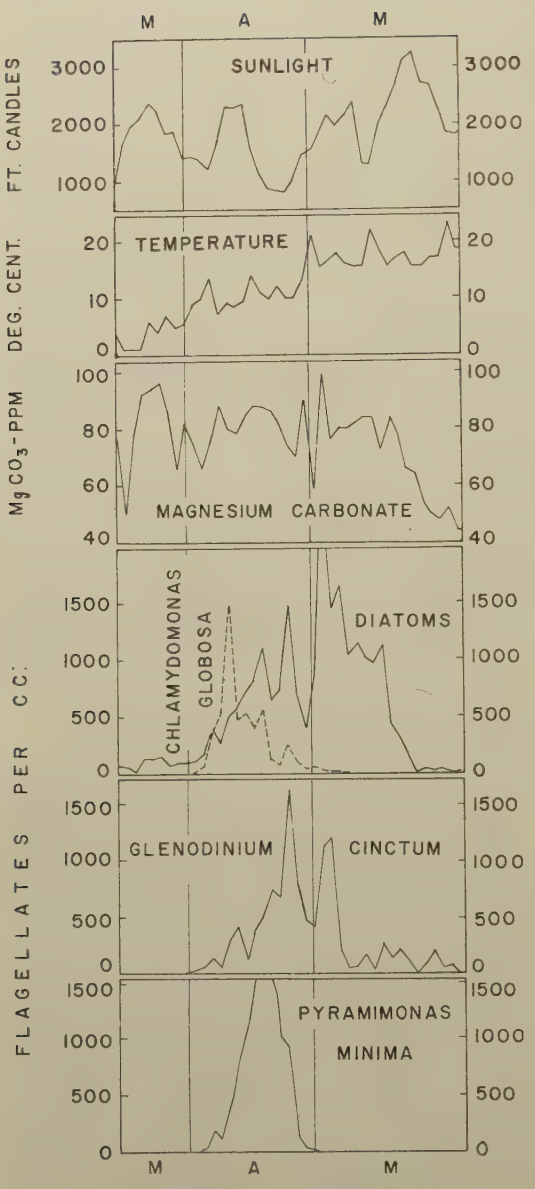

FIG. 8. Succession of spring phytoplankton. Sunlight curve is a five-point moving average; other curves plot unmodified data. Low sunlight favored *Glenodinium cinctum*, whereas greater sunlight and increased temperatures favored diatoms.

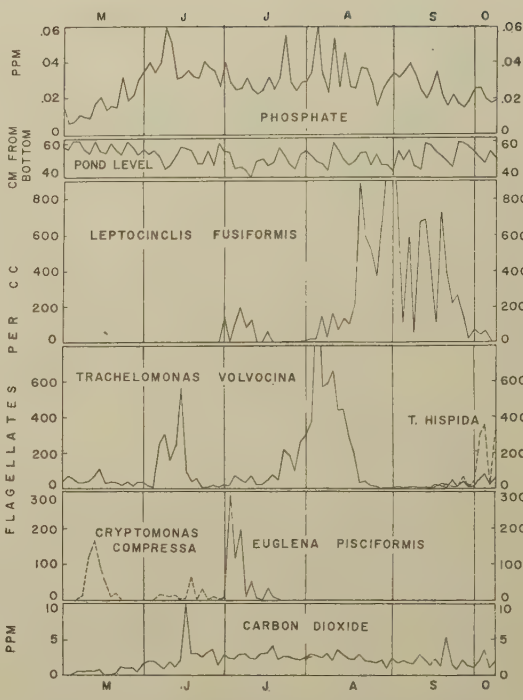

FIG. 9. Distribution of summer phytoplankton, mainly euglenoids. PO_4 and CO_2 are indicators of decomposition processes, which furnish substances required by euglenoids.

Glenodinium cinctum have been made on this pond. In December and January following the survey *G. cinctum* became the dominant organism. In the author's winter survey of 1953–54 this species appeared in low numbers in December, and exhibited a population increase in late March and April before *Pyramimonas* and diatoms established themselves. In Wang's (1928) survey *G. cinctum* showed a surge in September and October, and appeared in low numbers in January and April.

The temporal distribution of *Glenodinium cinctum* suggests that several factors may influence its distribution. The disappearance of the organism in summer suggests that it cannot tolerate an environment containing high concentrations of ammonia and phosphate, but the appearance of at least a few individuals over the rest of the year suggests that the species possesses a wide tolerance to both temperature and chemical environment. The abundance of *G. cinctum* in early spring and the autumn suggests that light and temperature may profoundly influence its distribution. The numerical ascendency of the diatoms over *G. cinctum* in the spring of the survey may have been due to higher temperature which favors the rate of assimilation of light and was rein-

forced by the longer days. Increased light and temperature may be unfavorable for the dinoflagellate. The other data cited for *G. cinctum* support this interpretation.

Diatoms, belonging principally to the genera *Stephanodiscus*, *Tabellaria*, *Fragilaria*, and *Navicula*, were present in low numbers throughout the survey and exhibited a population surge in April and May. As noted above, increase in temperature and sunlight intensity appear to have favored their multiplication. The rapid decline of the diatoms in early May did not coincide with any factor except a rapid rise (0.01 to 0.02 ppm) in phosphate. Rodhe (1948) has shown that some diatoms (*e.g.*, *Asterionella*) possess only a moderate phosphate tolerance. Other substances might have been present, however, since the decaying organic matter producing the phosphate also released other substances.

About two dozen species of euglenoids, belonging mainly to the genera *Euglena* and *Trachelomonas*, comprised the summer population from June to the end of the survey. Two species dominated—*Trachelomonas volvocina* and *Leptocinclis fusiformis*—with a small population of *Euglena pisciformis* in June and a growing population of *T. hispida* in October (Fig. 9). *T. volvocina* was found from spring through autumn. Population increases of the species followed increases of phosphate but decreases of the pond level. *Leptocinclis* succeeded *T. volvocina* and fluctuated in the same manner except that two of its surges followed rainstorms. *Euglena pisciformis* increased in July during a period of high ammonia concentration but moderate phosphate concentration. *T. hispida* increased in October when ammonia and phosphate were at lower concentrations, and the flagellate continued into November as the dominant organism.

Provasoli and Pintner (1955) have shown that *Trachelomonas* can tolerate one-hundred times the concentrations of ammonia and phosphate reported here. In this pond the low concentrations of phosphate might exercise an inhibiting effect on *T. volvocina* and *Leptocinclis*. *E. pisciformis* and *T. hispida*, on the other hand, might be able to reproduce at lower concentrations of phosphate. The decaying organic matter in the pond producing the phosphate also produced simple organic compounds (acetate, amino acids) and vitamins. The lowering of the pond level concentrated all substances in the water. The numerical increase of the euglenoids during these periods, and the two *Leptocinclis* surges following rainstorms (mixing periods) suggest that concentration and circulation of nutrients may be an important factor. Phosphate and ammonia may set minimal survival conditions, and growth above this level may depend on the supply of other nutrients.

The continuance of *T. hispida* into the autumn suggests that light and temperature may influence its distribution.

Zooplankton

Planktonic Sarcodina were too few in numbers and too discontinuous in their distribution to attempt any comparisons other than that they were usually found in the cooler portions of the year. Most belonged to the Heliozoan species *Actinophrys sol*, *Acanthocystis mimetica*, *Lithocolla globosa*, and *Heterophrys glabrescens*.

Rotifers were present in low numbers in autumn and spring. They exhibited a population surge in May, and showed fluctuations during the summer. Four species dominated: *Anuraeopsis fissa*, *Brachionus calyciflorus*, *Mytilina trigona*, and *Lepadella patella*. All four possess malleate jaws and are particulate feeders. The May surge was too great to show correlations with any environmental factors and probably represents the seasonal reproductive period, but fluctuations during the summer were correlated directly with phosphate and turbidity fluctuations. As noted earlier, the twofold increase of turbidity in summer, with decreased plankton (compared to spring) indicated a large amount of detritus in the water. Hence food supply appears to be a major factor determining the group's distribution. The lack of phytoplankton predation by rotifers supports Riley's (1940) and Pennak's (1946) conclusions that most zooplankton feed on bacteria and organic debris.

The majority of ciliates appeared in three population surges: a January increase of

small Holophryidae belonging to the genus *Urotricha*, a spring increase of *Phascolodon vorticella* and of hypotrichs belonging to the family Oxytrichidae, and a summer increase of large Holophryidae. These three surges reflected different nutritional habits.

The January increase (Fig. 6) of *Urotricha synuraphaga* and *U. globosa* followed a surge of chrysomonads, the bodies of which were easily discernible in those of the ciliates.

The first spring ciliates, consisting of Oxytrichidae, appeared as the *Chlamydomonas* population was rapidly declining. These hypotrichs were succeeded by the gymnostome *Phascolodon vorticella*, which increased when the *Gonium* population had greatly declined (Fig. 7). Both groups of ciliates were about equally numerous at surface and bottom, and their bodies were full of grass-green inclusions. The same predator-prey relationship of these ciliates was observed in the author's winter survey of 1953–54.

Summer ciliates, belonging mainly to the genus *Holophrya*, appeared after the rise in ammonia in late May, and exhibited extreme fluctuations of numbers, which usually followed those of phosphate and turbidity. Heavy rainfall sometimes drove the surface population below, but otherwise surface and bottom populations were about equal in numbers. Increases of ciliates showed little agreement with increases in sunlight. These observations parallel the observations on rotifers, and indicate that bacteria and organic debris constitute the principal food supply. The low numbers of summer ciliates, of the free swimming type, compared with the very numerous flagellates, together with the high ammonia and phosphate concentrations indicate considerable organic decomposition, particularly when this survey is compared with Wang's (1928) survey. This suggestion has been made by Baines *et al.* (1953).

The appearance of ciliates on cloudy as well as sunny days and the lack of agreement of their trend with the sunlight trend (Figs. 3 and 5) fail to support Richards' (1929) hypothesis that sunlight is an important factor in increasing ciliate numbers; rather, their distribution appears to confirm Noland's (1925) conclusion that the nature and amount of available food is the controlling factor.

DISCUSSION

Sampling criteria

Sudden changes can and do occur in a body of water in short periods of time. Examples in this survey are seen in the sudden rise and fall of chemical factors, such as ammonia, nitrite, and dissolved oxygen, and the fluctuations of populations, such as those of *Chlamydomonas incerta* and *Leptocinclis fusiformis*. These fluctuations emphasize the need of detailed studies on limited natural environments over a long period of time as a prerequisite to a better understanding of the ecological relationships of planktonic organisms. Although only a few of the many factors were measured in this survey, frequent sampling has yielded associations not revealed by more random methods.

This survey underscores Hammann's (1952) call for a revision of the "saprobic" system of Kolkwitz and Marsson. The disappearance of *Dinobryon* in an "oligosaprobic" environment due to a slight increase in phosphate, and the appearance of *Cryptomonas* in June in a "mesosaprobic" environment (during a period of low ammonia concentration), call for a more precise definition of protozoan habitats in terms of dissolved gases, nitrogen and phosphorus compounds, minerals, organic substances, pH, and bacteria.

Influence of chemical factors

The distribution of organisms in a body of water depends on a variety of factors: physical factors, inorganic nutrients (P, N, Ca, Na, dissolved gases, etc.), organic materials (acetate, amino acids), trace elements (Zn, Co, etc.), vitamins (particularly thiamin and B_{12}), and substances produced by organisms. Although only a few of the first two groups were measured in this survey, some inferences may be made about certain other factors.

Amount of phosphate is an important factor in determining which phytoplankters may appear. Rodhe (1948) has grouped

phytoplankton according to phosphate requirements: low, represented in this pond by chrysomonads; medium, represented by *Cryptomonas*, *Chlamydomonas*, and diatoms; and high, represented by many euglenoids. This classification depends upon the "critical" concentration of 0.02 ppm of phosphate, which represents the maximum concentration that organisms of the first group can tolerate, and the minimal concentration that organisms of the third group require in order to reproduce. Organisms in the second group can grow in waters containing as little as 0.005 ppm of phosphate and also in waters containing several times this amount; hence the second group overlaps the other two. The utilization of phosphate within this range may depend upon other factors. For example, Provasoli and Pintner (1953) found that the amount of phosphate necessary to produce maximal growth of *Cryptomonas ovata* in culture depends upon the amount of magnesium sulphate present. Rodhe (1948) has shown that *Asterionella formosa* requires one-hundred times more phosphate in culture than in nature to produce maximal growth, but that when the organism is transferred to (filtered) lake water, the addition of only one per cent of the *in vitro* phosphate requirement produces maximal growth. This striking discrepancy in phosphate utilization suggests that natural waters contain one or more factors that facilitate the use of phosphate by phytoplankton. Hence the phosphate need varies among phytoplankters, and some organisms have a narrow optimal phosphate range.

Growth factors may be very influential. The increase of winter phytomonads during a period of presumably low concentration of nutrients may be due to their autotrophic nature. They are not (generally) dependent on growth factors or carbon sources other than carbon dioxide, in contrast to the auxotrophic cryptomonads and chrysomonads which preceded them. The heterotrophic tendencies of euglenoids restricts many of them to the warmer portions of the year, when decomposition processes furnish needed vitamins and organic compounds.

Degrees of sensitivity to concentrations might be seen in cryptomonads and chrysomonads, both described as "oligosaprobic" organisms. The chrysomonads exhibit low tolerance to phosphate and osmotic pressure, but the cryptomonads, while also requiring a dilute environment, must not be as sensitive, since they preceded chrysomonads in this pond, and also appeared in May and June during periods of high phosphate concentration.

Biotic substances

Le Fevre *et al.* (1952) on the basis of *in vitro* experiments with algae showed that phytoplankters can produce at least two kinds of substances—those that enhance and those that inhibit growth, the latter being either auto- or heteroantagonistic or both. Consequently the workers explained succession of species thus: under favorable conditions a species multiplies rapidly and eliminates the greater part of other organisms, save for a few specially resistant individuals; then the dominant species becomes inhibited by autoantagonism and declines; during the decline a favorable moment is reached for a previously inhibited species, which then proliferates rapidly and succeeds the first species.

Rice (1954), on the basis of similar *in vitro* experiments, showed that pond water that had supported a *Pandorina* surge contained a thermolabile substance inhibitory to *Chlorella* and *Nitzchia*. Proctor (1957) isolated from plasmolyzing *Chlamydomonas reinhardi* cells a fatty acid inhibitory to *Hematococcus*.

The explanation of Le Fevre *et al.* accounts for the sporadic individuals of species like *Trachelmonas volvocina* appearing in early spring and late autumn under conditions not typical of those enjoyed by the summer surges of the species. The low numbers of flagellates in March (Fig. 5) between winter and spring populations may be due to heteroantagonistic substances.

Observations on the winter phytomonads in this pond parallel those of Proctor. He found that after a population of *Chlamydomonas* reached its peak, cells began to plasmolyze and release a fatty acid. The appearance of predaceous ciliates in March and April after the peak of the phytomonad

population parallels this phenomenon (Fig. 7). This relation was also observed in the author's 1953–54 survey. Biotic substances might also explain the appearance of *Urotricha* toward the end of the winter *Synura* surge. (A concentrated sample of *Synura* gives off a noticeable odor of ripe cucumbers.) How are the predators informed of their prey?

The large Holophryidae appeared in this survey with the sudden increase of ammonia, an indication of decomposition processes. These ciliates are mainly bacteria and detritus feeders and their appearance may be explained by the experimental work done on *Colpoda*. Thimann and Barker (1934) showed that a weak acid in plant tissues produces excystment in concentrations as dilute as a few parts per million; and Taylor and van Wagtendonk (1941) recorded that removal of bacteria caused encystment, but that organic solutes were related to the encystment process.

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The Accumulation of Y^{90} from an Equilibrium Mixture of Sr^{90}-Y^{90} by *Artemia salina* (L.)[1]

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ABSTRACT

Artemia salina (L.) was chosen as a representative organism in the second trophic level for studying one phase of the process of transfer of radionuclides through a marine food chain. Adult *Artemia* were cultured in filtered sea water which contained Sr^{90}-Y^{90}. They were not fed during the experiment which lasted 92 hours. They were removed at intervals, washed, and their radioactivity determined with an end-window counter. At 16 hours the concentration of radioactivity in the *Artemia* was about 40 times that of an equal weight of sea water. The radioactivity decayed at a rate which was similar to the decay rate of Y^{90}. It was estimated that only about 5 per cent of the activity remaining at secular equilibrium was due to Sr^{90}, the remainder being Y^{90}. This biological fractionation of Sr^{90}-Y^{90} may affect the transfer of these hazardous nuclides through food chains leading to human food. The fractionation of these nuclides by laboratory glassware is briefly discussed.

It has become increasingly apparent that in order to determine the biological hazards of radioisotopes from reactor wastes and from fallout we must increase our knowledge of both the rate of accumulation and the maximum concentration in the ecosystems involved. With such information we can more intelligently assess the importance of these isotopes in the biology of the individuals. It has been shown (Boroughs *et al.* 1956a, 1956b) that strontium and yttrium fed to fish are rapidly excreted. It has also been found that a relatively small amount is accumulated from the water. However, in all instances the amount retained seems to be dependent upon a number of factors, *e.g.*, species, age, food habits, and perhaps sex. It also appears that some organisms actually discriminate against one isotope in preference for another when both are present in the medium (Rice 1956). It is this effect which may reduce the concentration of isotopes such as Sr^{90} at various trophic levels.

Since the planktonic crustacea form a large percentage of the food at the lower trophic levels in the marine environment, they may serve as a means of transferring isotopes from the phytoplankton to the smaller carnivores. It was the purpose of the present work to determine the amount of Sr^{90} and Y^{90} accumulated by the brine shrimp, *Artemia salina* (Linnaeus), when these isotopes are present in sea water.

METHODS

All glassware used was freshly washed with concentrated H_2SO_4, detergent, and rinsed several times in distilled water. About 61.2 μc Sr^{90}-Y^{90} from Oak Ridge was diluted to 100 ml with sea water filtered through a glass fiber filter which retained particles of bacterial dimensions (0.1 microns). From this solution five 0.5-ml aliquots were pipetted onto aluminum planchettes and dried. Twenty-five adult *Artemia salina* were added to the sea water containing the Sr^{90}-Y^{90} mixture. Five brine shrimp were immediately removed from the solution, drained, and washed three times with distilled water. Preliminary experiments showed that very little radioactivity was lost by repetitive washing, and that distilled water and sea water washes were equivalent. The *Artemia* were then blotted with filter paper, arranged uniformly on an aluminum planchette, and dried under a heat lamp.

At intervals of 16, 40, 64, and 92 hours

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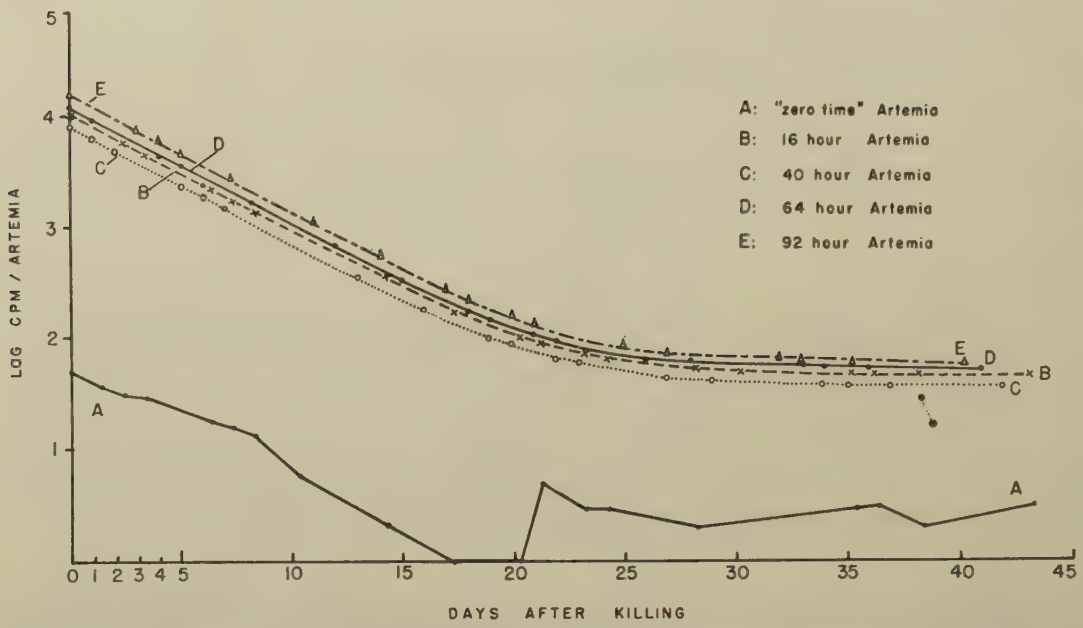

FIG. 1. The radioactive decay of Sr^{90}-Y^{90} in *Artemia* showing the accumulation of Y^{90} rather than of Sr^{90}.

later, five *Artemia* were removed and prepared as above. During this time the brine shrimp were not fed. When each group of brine shrimp was removed, 0.5-ml aliquots of the sea water were also pipetted onto aluminum planchettes and dried.

As each set of samples was prepared it was immediately counted on a commercial scaler using an end-window Geiger-Müller tube. All samples were counted daily in order to follow the radioactive decay, and appropriate corrections were made for coincidence when necessary. Owing to the high cpm, all samples were counted on the third shelf of a lead support, or approximately 4 cm from the Geiger-Müller tube.

Strontium^{90} from Oak Ridge is an equilibrium mixture of Sr^{90} and Y^{90}. Strontium^{90} has a half-life of about 28 years, and has a beta radiation with a maximum energy of 0.61 Mev. The daughter product of Sr^{90} decay is Y^{90} which has a half-life of about 2.5 days and has a beta radiation with a maximum energy of 2.18 Mev. At equilibrium the number of counts recorded is due to both the Sr^{90} and its daughter Y^{90}. If absorbers are used, or if there is a biological fractionation of the isotopes, the Geiger-Müller tube will count all particles entering it, but because more high energy particles enter the tube per disintegration than low energy particles, it will be more efficient for Y^{90}. A lapse of 10 half-lives of Y^{90} or about 25 days re-establishes the secular equilibrium of the isotopes. Thus it was necessary to count the samples at intervals for 25 days in order to be able to tell whether Sr^{90} or Y^{90} had been removed from the solution.

RESULTS AND DISCUSSION

Figure 1 is a semi-log plot of the radioactive decay of *Artemia* exposed to Sr^{90}-Y^{90} sea water for 0, 16, 40, 64, and 92 hours. It can be seen that when the brine shrimp were removed, the cpm/*Artemia* ranged from 8,200 to 12,000, but 43 days later it had dropped to 35–56 cpm/*Artemia*. From these curves several conclusions may be drawn. According to Curve A, there was no *immediate* binding of either isotope by the shrimp, but Curve B indicates that a large amount of radioactivity was taken up by the shrimp during the first 16 hours. The zero-time *Artemia* had about 55 cpm/*Artemia* when the samples were counted for the first time. These shrimp averaged 10.3 mg, so that 1 gram of *Artemia* would take up about 5,335 cpm. The 16-hour *Artemia*, on

the other hand, had about 10^6 cpm/gram. One gram of sea water had about 25,000 cpm. These numbers show that the *Artemia* which were merely dipped into the water did not absorb much of the radioactivity present, but that the 16-hour *Artemia* actually concentrated it about 40 fold (10^6/25,000).

The decay rate indicates that about half the initial radioactivity disappeared in 2.5–3.0 days, and this time is approximately equal to the half-life of Y^{90}. The high level of radioactivity, therefore, is due to the Y^{90} component, and the small residual radioactivity is due to the Sr^{90}.

The ratio of cpm/g *Artemia* to cpm/g sea water we call the concentration ratio. Table 1 shows the concentration ratios of the various samples at equilibrium. It can be seen that there is no significant change between the amount of Sr^{90} picked up after 16 hours and that picked up after 92 hours in the medium. On a weight basis one can therefore expect adult brine shrimp in a medium containing Sr^{90}-Y^{90} to accumulate about 13 per cent of the total available nuclides. At equilibrium, only half the disintegrations are due to Sr^{90}, but because of the difference in counting efficiency for the two different energy beta particles, it is thought that only about 5 per cent of the radioactivity at equilibrium is due to the Sr^{90}.

The concentration ratio at equilibrium is roughly the same regardless of the time the *Artemia* spent in the radioactive water (up to 92 hours). However, these results might be changed by longer periods of exposure, or by differences in age and sex of the animals.

It is apparent from the work of Chipman *et al.* (1955, 1956) that age is an important factor in determining the amount of Sr accumulated from solution. These workers exposed *Artemia salina* nauplii to Sr^{89}-Sr^{90}-Y^{90} in sea water for 24 hours. Table 2 shows their data calculated on the same basis as in Table 1. These data suggest that young *Artemia* accumulate more Sr from sea water than do adults. The higher concentration ratios may mean that *Artemia* which spend their entire lives in sea water containing Sr^{90}-Y^{90} will have more than the five per cent Sr^{90} we found with adults.

TABLE 1. *Concentration ratio of Sr^{90}-Y^{90} in adult* Artemia salina

Sample	cpm/g	Concentration ratio	Mean
Sea water	36,000		
Artemia—16 hrs	4,600	0.13	
Artemia—40 hrs	3,500	0.10	0.13
Artemia—64 hrs	4,900	0.14	
Artemia—92 hrs	5,600	0.16	

TABLE 2. *Concentration ratio of Sr^{89}-Sr^{90}-Y^{90} in* Artemia salina *nauplii*

Exposure time (hrs)	Sea water cpm	*Artemia* cpm	Concentration ratio
0.5	11	4.7	0.43
1	11	6.5	0.59
2	11	6.5	0.59
4	11	7.2	0.65
7	11	8.5	0.77
24	11	7.8	0.71

The amount of Sr^{90} taken up may also be increased if the Sr^{90} is present in phytoplankton which the *Artemia* eat.

Artemia, therefore, discriminate against the long-lived Sr^{90} in preference for the short-lived Y^{90}. It is possible that through such fractionation the more hazardous Sr^{90} may be lost by the time the higher trophic levels are reached. On the other hand, some organisms may concentrate strontium almost exclusively from an equilibrium mixture. Thus, Rice (1956) found that 90 or more per cent of the radioactivity in 10 species of algae was due to yttrium, but that *Carteria* accumulated only strontium. Such organisms as *Carteria*, therefore, may be a considerable source of contamination which can be transferred up the food chain. It is apparent that the loss of an isotope in one food cycle may promote the enrichment of this isotope in another cycle.

The radioactivity of the sea water samples was also followed from day to day. Figure 2 shows that at zero time the water had an activity of 15×10^3 cpm per 0.5 ml. Water samples which were removed 40, 64, and 92 hours later, however, had an activity of 12.5 to 13.5×10^3 cpm per 0.5 ml. At equilibrium the radioactivity in all the water samples had increased to 17–18.5 $\times$ 10^3 cpm per 0.5 ml. This was an increase in radioactivity of 20–60 per cent above that found when the samples were first counted.

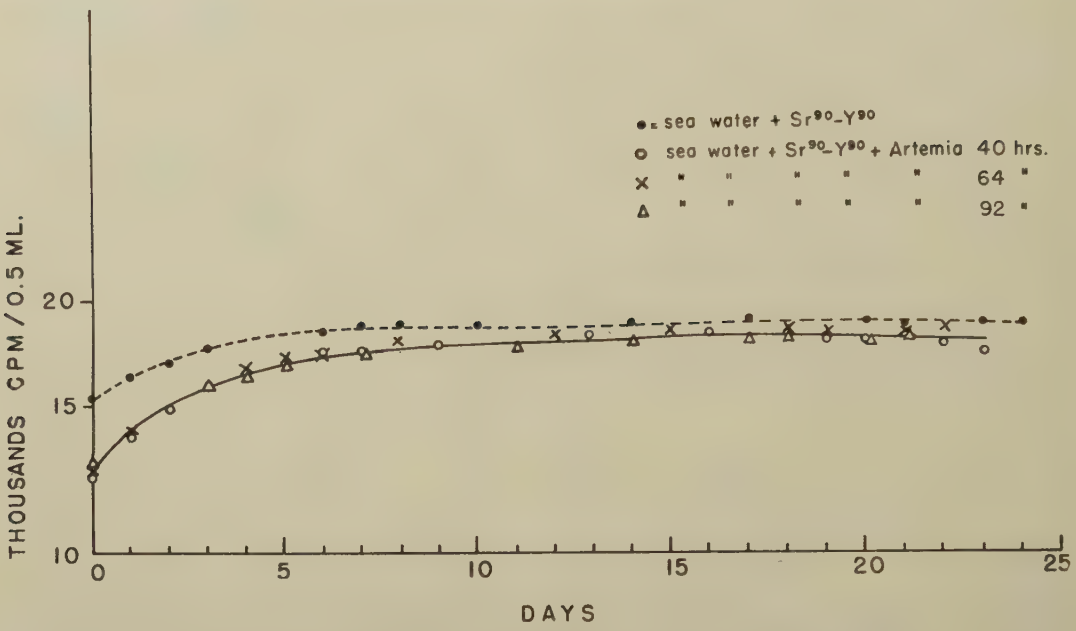

FIG. 2. The increase in radioactivity of the sea water medium, showing the build up of Y^{90} as a result of fractionation of the original Sr^{90}-Y^{90} mixture.

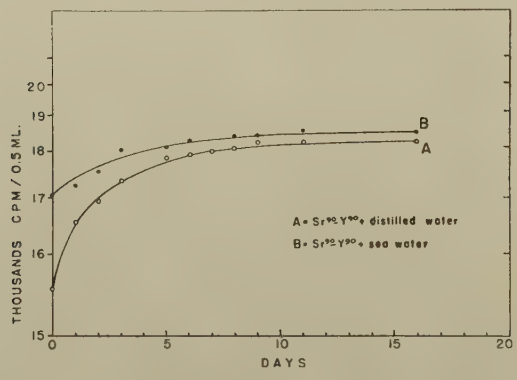

FIG. 3. The adsorption of Y^{90} by glassware from distilled water and sea water solutions of Sr^{90}-Y^{90}.

Of particular interest is the fact that the number of counts/minute for all the samples at equilibrium was approximately the same irrespective of the number of counts/minute when the samples were prepared (*cf.* 0^t dose and 92^h dose). This rise in activity implies that in all the samples some Y^{90} had been removed from the original equilibrium mixture.

It must be pointed out that using an equilibrium mixture of Sr^{90}-Y^{90} for such studies as these presents many problems, because it is difficult actually to assess the role of each isotope in the biological sense. This difficulty is due to the physical and chemical nature of the mother and daughter products.

Strontium is very likely ionic in sea water, but yttrium is probably at least partially colloidal. Strontium is bivalent and yttrium trivalent, and there is a significant difference in the size of their ionic radii. These differences mean that the biological binding sites for the two elements may be wholly different, but the sites may also overlap. If the sea water planchettes had been counted only at the time they were made, one would never suspect that there would be a significant change in the amount of radioactivity over a short time interval. Since Sr^{90} has a half-life of about 28 years, one would expect a constant count rate over a period of 30 days. However, when the isotope from Oak Ridge was pipetted into the sea water, Y^{90} was immediately lost, most likely by being adsorbed onto the glass. This means that each step in pipetting removed more yttrium from the mixture and upset the equilibrium. To test this hypothesis Sr^{90}-Y^{90} was added to flasks con-

taining sea water or distilled water. Figure 3 shows that more Y^{90} was adsorbed from distilled water than from sea water.

Thus it appears to us that in working with such isotopes which may have a tendency to become adsorbed onto surfaces, much care must be taken in the interpretation of data, since considerable variation may occur depending upon the time intervals concerned. Furthermore, it is apparent that it is very difficult to work with an equilibrium mixture of Sr^{90} and Y^{90} using ordinary glassware.

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Annual Fluctuations in Sulphate and Bicarbonate Hardness in Ponds

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ABSTRACT

In four ponds studied, sulphate in solution was high in spring, low in autumn, while bicarbonate was low in spring, high in autumn. The fall in sulphate was probably due to reduction to sulphide which became locked up in the mud as ferrous sulphide. The fluctuation in bicarbonate was partly due to the removal of CO_2 during photosynthesis and partly to the action of sulphuric acid when the sulphide was oxidized in winter.

INTRODUCTION AND METHODS

In the course of a study of the annual fluctuations of the chemical composition of the water in a number of ponds, Tucker (1958) showed that there was a tendency for the calcium content to fall in summer, while the titration against standard acid (alkalinity) tended to rise. This tendency was more marked in some ponds than others (Fig. 1), and Tucker concluded that the fluctuations were greatest in productive ponds, normally characterized by a high content of dissolved organic matter.

At first sight it seems likely that an important factor in these fluctuations is the higher level of photosynthetic activity in summer. According to Rabinovitch (1945–56) the probable action of algae during photosynthesis is the removal of CO_2 from solution in the water, and the equilibria which are likely to involve the titratable base are:

$$CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{--} \quad (1)$$

$$CaCO_3 \rightleftharpoons Ca^{++} + CO_3^{--} \quad (2)$$

$$CO_3^{--} + CO_2 + H_2O \rightleftharpoons 2HCO_3^- \quad (3)$$

The effect on (1) of the removal of CO_2 from the system is a fall in the hydrogen ion concentration, and in the concentration of carbonate and bicarbonate ions. The effect on (3) is the splitting off of carbon dioxide from the bicarbonate ions, and the precipitation of calcium carbonate according to (2). There will therefore be a fall in the concentration of bicarbonate and calcium. The total effect is that the pH rises and the amount of titratable base decreases.

Thus the fall in calcium concentration can be explained by the photosynthesis of the plants, but the rise in concentration of bicarbonate is not in accordance with expectation. The present paper is the record of an attempt to verify an alternative hypothesis, arrived at after helpful discussions with Dr. F. J. H. Mackereth of the Ferry House Laboratory of the Freshwater Biological Association. This is, that sulphide ions derived from the bottom deposits under anaerobic conditions in summer are oxidized to sulphuric acid in winter, converting a proportion of the calcium bicarbonate to sulphate. This would lead to a fall in the titer to standard acid, and if the sulphate were reduced to sulphide when the pond became anaerobic again in summer, the calcium ions could take up bicarbonate again.

Tucker's observations were therefore repeated on certain ponds, and in addition estimates were made of sulphate and oxygen concentration in the water. The oxygen concentration was measured in samples taken from a few cm above the mud surface, but other determinations were made on samples from the surface of the water at the edge of the pond. Calcium was determined by versenate titration, using ammonium purpurate as indicator (Betz and Noll 1950), and total alkalinity by titration to the end point of xylene-cyanole methyl orange with standard acid. Sulphate was determined by precipitation with benzidine hydrochloride (Owen 1936).

DESCRIPTION OF THE PONDS STUDIED

The ponds were chosen to represent soft water and hard, very shallow basins and deeper basins.

1. *Heath pond.* The water is soft (calcium ca. 8 mg/L), acid (pH ca. 5.8), and colored by humic acids. *Sphagnum* grows at the edges. The surface area is about 10,000 m^2 and the depth ca. 1.5 m.

2. *Hammond's pond.* This is a shallower version of the previous example, the mean depth being ca. 0.6 m. Both ponds are situated in country dominated by pine and birch. In this case the calcium content is about 7 mg/L and the pH about 6.3.

3. *Leech pond* has harder water (calcium ca. 30 mg/L) nearly neutral in reaction. The basin has a surface area of about 4,000 m^2 and a depth of 1.3 m in the center. It is closely surrounded by mixed deciduous trees, and in summer much of the surface of the water is covered by *Lemna.* The bottom mud is highly organic.

4. *Egham pond.* A shallow pond of the harder water type, it has about the same surface area as Leech, but the depth is only about 0.6 m. It is situated in pasture land, and large areas of the pond are colonized by *Potamogeton natans.*

OBSERVATIONS AND DISCUSSION

The data for calcium concentrations, bicarbonate, and sulphate are given in Table 1. From a consideration of Tucker's figures for pH, confirmed by my own observations, it is clear that the alkalinity may be interpreted as being due entirely to bicarbonate (Moore 1939). Fluctuations in sulphate and bicarbonate in Egham and Leech ponds are illustrated in Figure 2. These show clearly that in both ponds the sulphate content of the water was rising steeply in January, while the bicarbonate content was falling, and that the reverse was true in June. Thus the sulphate content is low in autumn and high in spring, while the bicarbonate content is high in autumn and low in spring. The changes seem to occur about one month later in Egham than in Leech pond. The oxygen content of the water near the bottom mud was 0–10% of saturation between June and October in both ponds, and this coincides

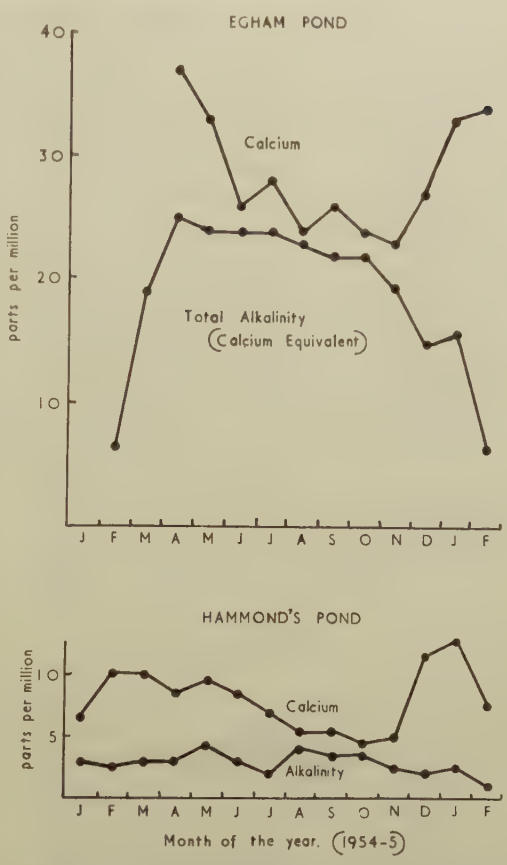

FIG. 1. Annual fluctuation in calcium and total alkalinity in Egham and Hammond's ponds, 1954–5. (After Tucker 1958.)

with the period of high bicarbonate and low sulphate content. The oxygen concentration rose steadily from November to February, which is the period of rising sulphate and falling bicarbonate concentrations. These facts support the suggestion that the sulphates of the water are derived from the sulphides produced during organic decay in the bottom deposits, by oxidation in winter and early spring, and that a proportion of these sulphates are reduced again when the pond becomes anaerobic in summer. Thus the calcium ions present are balanced by bicarbonate and sulphate ions in varying proportions, and if the amount of calcium present remained constant, we should expect a fall in bicarbonate ions which just balanced the rise in sulphate ions. To check this point the autumn and spring levels of the

TABLE 1. *Chemical data in m-equiv per litre*

	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Calcium												
Heath	0.31	0.31	0.31	0.35	0.38	0.35	0.37	0.32	0.30	0.40	0.27	0.32
Hammonds	0.35	0.28	0.36	0.34	0.36	0.25	0.26	0.22	0.21	0.29	0.18	0.22
Leech	1.02	1.02	1.22	1.20	1.27	1.24	1.65	1.43	1.54	1.44	1.43	1.44
Egham	1.05	1.06	1.17	1.10	1.08	1.04	1.22	1.11	1.11	1.13	1.05	1.18
Bicarbonate (= total alkalinity)												
Heath	0.24	0.16	0.14	0.05	0.06	0.06	0.07	0.06	0.06	0.06	0.11	0.19
Hammonds	0.12	0.10	0.08	0.08	0.06	0.06	0.07	0.10	0.12	0.22	0.19	0.15
Leech	0.96	0.88	1.06	1.06	0.84	0.56	0.61	0.62	0.64	0.72	0.94	0.98
Egham	1.28	1.12	1.16	1.10	1.16	0.84	0.86	0.86	0.92	1.10	1.20	1.22
Sulphate												
Heath	0.44	0.34	0.44	0.64	0.76	0.80	0.64	0.70	0.68	0.77	0.49	0.30
Hammonds	0.54	0.34	0.42	0.48	0.56	0.32	0.30	0.29	0.34	0.18	0.10	0.06
Leech	0.90	0.86	0.76	1.06	1.40	1.68	1.67	1.90	1.94	2.02	1.30	1.20
Egham	0.18	0.18	0.18	0.11	0.46	0.70	0.70	0.60	0.56	0.34	0.12	0.06

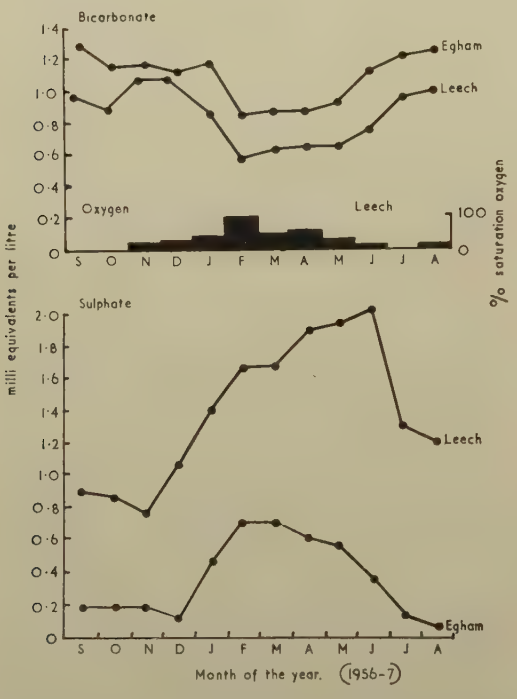

FIG. 2. Annual fluctuations in bicarbonate and sulphate in Leech and Egham ponds, with the fluctuations in oxygen content of the water just above the mud in Leech pond, 1956–7.

two ions have been calculated by taking the mean of September, October, and November readings on the one hand, and February, March, and April readings on the other (Table 2).

Clearly the rise in sulphate content is much greater than the corresponding fall in

TABLE 2. *Seasonal changes in bicarbonate and sulphate content, in m-equiv per litre*
For method of calculation see text.

	Bicarbonate			Sulphate		
	Autumn	Spring	Diff.	Autumn	Spring	Diff.
Leech	0.97	0.60	−0.37	0.84	1.75	+0.91
Egham	1.19	0.85	−0.34	0.18	0.70	+0.52

bicarbonate. This will be the case if at the same time of the year bicarbonate ions are coming into solution as a result of some other process. It has already been mentioned that on theoretical grounds one expects photosynthesis to bring about formation of calcium carbonate in summer[1] and its reconversion to bicarbonate by the action of carbonic acid in winter. The magnitude of any such reaction is shown by the annual fluctuation in the amount of calcium in solution. In Leech the October-November value was 1.02 m-equiv/L and the mean of March, April, and May was 1.54 m-equiv/L. We may therefore infer that in the intervening period calcium and bicarbonate ions of 0.52 m-equiv/L were brought into solution. When the same calculation is made for Egham, there is a difference of 0.10 m-

[1] It is just possible that the solubility product of calcium carbonate is not exceeded in the softer waters in summer, in which case the annual fluctuations of calcium may be due to the adsorption of calcium ions onto humic substances in the mud at the time when calcium sulphate is being reduced, and their liberation when sulphide is being oxidized.

equiv/L between autumn and spring values of calcium. The situation in Leech and Egham would therefore seem to be that between November and February there is a substantial increase in sulphate ions in the water, and the calcium ions with which these are associated are derived partly from the calcium bicarbonate already present in the water (accompanied by the formation of carbon dioxide and water) and partly from the calcium carbonate previously precipitated during photosynthesis (or adsorbed, see footnote 1) by the action of carbonic acid. In Leech the 0.91 m-equiv/L of sulphate is balanced by 0.37 m-equiv/L of calcium derived from bicarbonate and 0.52 m-equiv/L derived from the carbonate. The remainder of the balance is probably accounted for by a change in magnesium concentration.

The time lag of the events in Egham compared with those in Leech can perhaps be accounted for by the fact that Egham has a dense growth of rooted vegetation to the center, which might be expected to delay the circulation of the water between the mud surface where the main chemical events take place, and the surface of the pond where the samples were taken.

Turning to Heath pond and Hammond's pond, we notice that the levels of calcium and bicarbonate are much lower at all times than in the other ponds. On the other hand the sulphate content of Heath pond is at most times higher than in Egham. A calculation made on the same basis as before gives the results shown in Table 3.

Clearly the sulphate and bicarbonate are balanced by a considerable amount of some ion other than calcium, and although complete analyses of the water were not attempted, it is probable that the other cations are chiefly magnesium and sodium. The analyses of Gorham (1956) include figures for bog pools in which the importance of sodium and magnesium compared with calcium in these soft waters is clearly shown. In Hammond's pond the winter rise in sulphate content of the water was less well marked than in Heath, and the concentrations of ions involved are so low that it is hardly worth while attempting to draw up a balance sheet. It does however appear that in the soft waters of Heath and Hammond's as well as in the harder waters of Leech and Egham there is an annual change in the balance between sulphate and bicarbonate ions, the magnitude of the change being greater in the deeper waters of Leech and Heath. Another point worth noting is that the average sulphate content of the ponds on heath country is several times greater than the bicarbonate content, *i.e.*, the hardness is mainly permanent hardness whereas in the ponds on more calcareous soil the sulphate content is about equal to the bicarbonate content. In the latter ponds the permanent hardness is more in evidence in the spring, and the temporary hardness in the autumn.

The annual fluctuations in sulphate content and alkalinity are given for Esthwaite water by Mortimer (1942). His samples were taken from just above the mud surface, and he showed that when the water was anaerobic there was a steady decline in the sulphate content, accompanied by a steady rise in alkalinity. At the overturn in September when the oxygen content of the hypolimnion rose sharply, the sulphate also increased sharply, and the alkalinity fell. Thus it appears that the sequence of events in the surface waters of the ponds investigated is comparable with that found in the hypolimnion, in a depth of 14 m, in Esthwaite water. Mortimer also studied the sequence of events in artificially aerated and in anaerobic tanks of water, both of which had a layer of mud on the bottom. He found that in aerobic conditions the sulphate content increased steadily over a period of five months, while in the anaerobic tank the sulphate content fell to zero in the same period. Conversely, the alkalinity fell to a low level in aerobic conditions, but rose steeply in anaerobic conditions. These experiments bring out the importance of dissolved oxygen as a factor controlling the

TABLE 3. *Seasonal changes in Heath Pond, in m-equiv per litre*

	Autumn	Spring	Difference
Bicarbonate	0.18	0.06	−0.12
Sulphate	0.41	0.71	+0.30
Calcium	0.31	0.35	+0.04

sulphate-bicarbonate balance. Comparing the ponds with the lake, it appears that whereas during summer stratification the reducing effect of the bottom mud is felt only in the hypolimnion of the lake, the whole of the pond water may be markedly influenced by changes taking place on the mud surface.

The importance of sulphates in the economy of lakes has been the subject of recent papers by Beauchamp (1953) and Ohle (1954). Beauchamp, referring to conditions in East Africa, considers that the small amount of sulphate present in Lake Victoria (0.8–1.8 ppm) restricts the growth of phytoplankton. The figures obtained in the present work for Hammond's and Egham ponds suggest that in August the concentration of sulphate is likely to be a limiting factor in plankton production. Ohle draws attention to the dual role of sulphates in fresh waters. In aerobic conditions they supply an essential element for protein metabolism, and in anaerobic conditions they provide the raw material for the chemosynthesis of sulphur bacteria, being reduced to hydrogen sulphide. This in turn brings about the reduction of ferric hydroxide, with the liberation of adsorbed phosphates, and is a critical point in the circulation of nutrients in the system. Hydrogen sulphide which escapes from the bottom deposits with bubbles of methane may escape to the atmosphere if the water is shallow, but is oxidized to sulphate on the way up if the water is deeper. It thus seems probable that in those waters where the fluctuation in sulphate content is most marked, there is a good supply of phosphates and other plant nutrients released from the bottom deposits, which will in turn lead to strong photosynthetic activity in summer, and a well marked precipitation of calcium ions at that time of year. This would explain Tucker's original observation that fluctuations in calcium and bicarbonate are most marked in the more productive waters.

In marine deposits the production of hydrogen sulphide from sulphate by bacteria under anaerobic conditions is well known (ZoBell, 1946), and according to Issatchenko (1929) the reaction of hydrogen sulphide with iron compounds, forming ferrous sulphide, is the cause of the characteristic color of the Black Sea. Likewise, the presence of bacteria capable of oxidizing sulphides has been demonstrated in many places, but there appears to be no record from salt water of an annual cycle of oxidation and reduction comparable to that described above.

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Determinations of the Solubility of Oxygen in Pure Water[1]

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ABSTRACT

A recently published revision of the absorption coefficients for oxygen in water has prompted a reinvestigation of the subject by a new microgasometric technique. A known volume of gas-free water (approximately 1 ml) was brought into contact with oxygen at constant pressure and temperature. The oxygen uptake and the amount of water were measured with a micrometer burette. The results obtained at 6°, 12°, 18°, and 24° C confirm the standard handbook values of Winkler and Fox to within ±1% of α.

The absorption coefficients for oxygen in pure water as given in the standard handbooks and tables (1952, 1955–1956) are based upon data obtained by Winkler (1889, 1891) and Fox (1909). Winkler made two series of determinations, one with a titrimetric method, the other with a gasometric method. In the latter he measured the amount of oxygen taken up by a known volume of gas-free water. The two sets of figures checked each other within a fraction of one per cent. Fox employed a gasometric method of the same principle as Winkler's. In the temperature range between 0° and 25°C Fox's data are 1–2% higher than those of Winkler.

The nitrogen solubility in water was determined by the same gasometric method as used for oxygen, and again Fox's data were some 2% higher than Winkler's. Winkler's results, both for oxygen and nitrogen have been checked at 20°C within 1% by Scholander *et al.* (1955), employing a micro volumetric gas extraction method.

Recently Truesdale *et al.* (1955), using the Winkler titration, published a revision of the solubility coefficients for oxygen in pure water and in sea water of varying salinity. The values, which are some 3% lower than those of Winkler, were confirmed by Wheatland and Smith (1955), using a volumetric gas-extraction method. Their water samples were prepared by the technique of Truesdale *et al.*, involving transfer of the equilibrated water samples under exposure to air. The revised solubility values have already won entrance in oceanographic literature (Richards and Corwin 1956). Inasmuch as Winkler's and Fox's large series of data were executed with a simple, strictly anaerobic, and apparently impeccable technique, it seemed desirable to check the proposed revision.

The method described below can be used to determine the solubility of any ideal gas in pure water with an accuracy of ±1% or better. It can be used for sea water or other saline solutions only provided these are made gas free by a technique which does not produce any change in water content or salt composition.

EXPERIMENTAL

Principle of method

A known volume of gas-free water is brought into contact with oxygen at constant pressure and temperature. The amount of gas taken up by the water is measured volumetrically.

Preparation of gas-free water

Water was freed from dissolved gases by boiling in a 1-liter pyrex flask (Fig. 1-I), letting the water vapor escape through a hole *A* in the glass stopper. After twenty minutes the water, still boiling, was transferred to a syringe through the siphon *B*. The hypodermic needle of a 10-ml all glass syringe was introduced through the rubber

[1] I am greatly indebted to Professor P. F. Scholander for encouraging discussion and advice and Mag. Scient. Helge Leivestad for aid in preparing the manuscript.

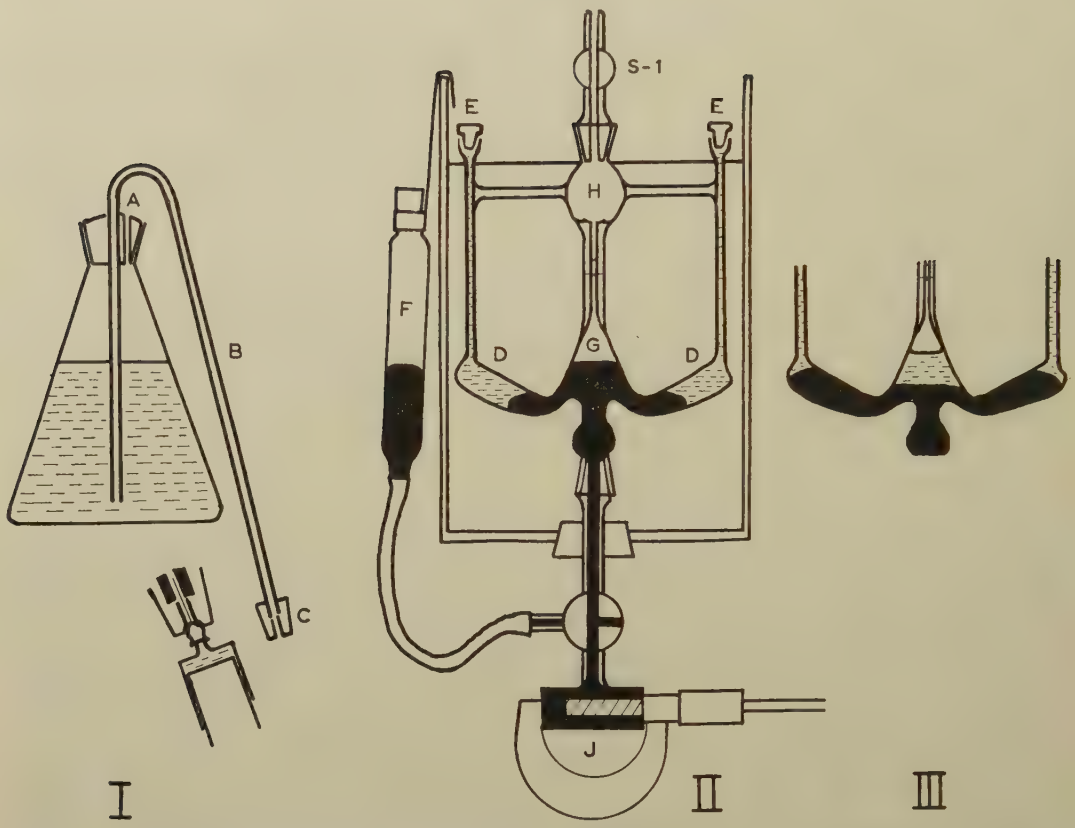

FIG. 1. I. Pyrex flask for preparing gas-free water by boiling. Detail shows the water being anaerobically transferred to the syringe. II. ½-cc gas analyzer for measuring the absorption of gas. III. Detail showing situation after water has been tilted into the absorption chamber from the side arms.

stopper *C*, and the syringe was filled by the weight of the water. Any gas bubbles from the syringe dead space were removed by repeated washing. When filling was complete, the needle was inserted into a rubber stopper and the syringe was stored plunger up.

Preparation of the absorption apparatus[2]

The absorption of oxygen in water was measured in a Scholander ½-cc gas analyzer (Fig. 1-II). The water bath of the analyzer was thermoregulated to within ±0.01°C, using a variac controlled light bulb as an external heat radiation source. The bath was well stirred by air bubbling, and the analyses were carried out in a thermoregulated room. The side arms *D* were flushed several times with gas-free water through the rubber stoppers *E*, care being taken that no air bubbles became trapped between the stoppers and the mercury. To prevent pressure differences in the micrometer, the mercury in the leveling bulb *F* was levelled with that in the absorption chamber *G*.

Procedure

The side arms *D* were filled with gas-free water from the syringe through the rubber stoppers *E*. The compensating chamber *H* was filled with water, and the mercury in the absorption chamber brought to the top of the capillary. A conventional gas transfer pipette, filled with oxygen, was seated on the capillary protected by the water seal. The gas meniscus was pulled down to the mark on the capillary, the micrometer

[2] The following brief description presupposes familiarity with the ½-cc analyzer. For details the original paper by Scholander (1947) should be consulted.

J connected with the leveling bulb, set to zero, and connected back to the capillary. The first reading (V_0) was taken on the micrometer. Approximately 11 mm oxygen on the micrometer were drawn into the absorption chamber. The transfer pipette was removed, and the water in the compensating chamber sucked off leaving a short water seal in the capillary to serve as index drop. The stopper S-1 was inserted, and the compensating chamber was flushed with oxygen by means of a thin cannula through S-1, which was then closed. The meniscus was adjusted to the mark, and the volume read (V_1), as soon as it had attained constancy, which generally took about two minutes. Water was then tilted in from the side arms (Fig. 1-III), gentle shaking started, and the index drop was kept at the mark by screwing in the micrometer. As soon as absorption was completed, which according to the temperature took some 20–40 minutes, the meniscus was adjusted to the mark and the volume read (V_2). The compensation chamber was opened, the stopper S-1 removed, and as the micrometer was screwed in the index drop was sucked off, the gas expelled, and the lower meniscus brought to the mark for the volume reading (V_3). The apparent volume increase represented by (V_3)-(V_0) is caused by increased hydrostatic pressure on the rubber stoppers E, and was therefore added to the volume of absorbed gas. The correction never measured more than 0.01 mm on the micrometer. Finally the volume of water was measured by replacing it with mercury from the micrometer, which was then read (V_4). This volume, which was measured in two steps, was commonly about 30 mm on the micrometer.

Calculation of the data

The volume of the absorbed oxygen reduced to STPD is:

$$V_g = (V_1 - V_2 + V_3 - V_0) \cdot \frac{273}{T} \cdot \frac{(p - h)}{760}$$

where p is the total pressure and h the water vapor pressure at the absolute temperature T. The volume of the water is: $V_w = V_3 - V_4$.

The absorption coefficient α is then:

$$\frac{V_g}{V_w} \cdot \frac{760}{p - h} = \frac{(V_1 - V_2 + V_3 - V_0)}{(V_3 - V_4)} \cdot \frac{273}{T}$$

It is seen that the measurement of α is independent of the pressure as long as this is constant.

Sources of error and accuracy of method

It was found that 15 minutes of boiling was sufficient to remove the last traces of detectable gas, and analyses showed that gas-free water could be stored in the syringe for several hours without being contami-

TABLE 1. *Absorption coefficients of oxygen in pure water*

	6.0°C	12.0°C	18.0°C	24.0°C
	0.04188	0.03673	0.03229	0.02901
	0.04186	0.03664	0.03231	0.02908
	0.04196	0.03674	0.03222	0.02898
	0.04186	0.03673	0.03235	0.02913
Mean values	0.04189	0.03671	0.03229	0.02905
Mean deviations	0.1%	0.1%	0.12%	0.14%
Maximum deviations	0.17%	0.19%	0.22%	0.28%

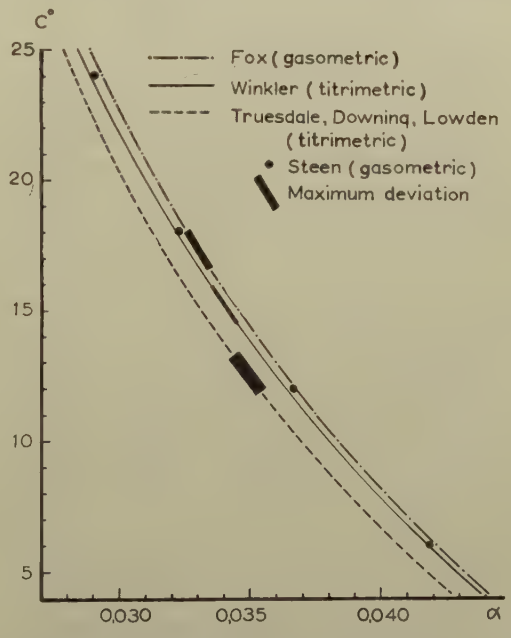

FIG. 2. Absorption coefficients found by various authors. Each point represents the mean value of four determinations (see Table 1).

nated with air. The gas used for saturation of the water contained 99.97% oxygen and 0.03% inert gases. There was no detectable loss of gas in the apparatus from oxidation of mercury or other sources.

When the absorption chamber was filled with oxygen and the compensating chamber with air, gas diffused through the index drop at a net rate of 0.005 − 0.01 mm on the micrometer per hour. This drift was eliminated by flushing the compensating chamber with oxygen. The equilibration of oxygen with the water took place through a gently agitated surface without bubble formation.

The accuracy of the determination of α is limited by the following factors. Temperature instability of ± 0.01°C gives an uncertainty of $\pm 0.1\%$ of α. The true temperature is known within ± 0.05°C, giving an uncertainty of $\pm 0.1\%$ of α. One reading on the micrometer is accurate to ± 0.0005 mm. Five readings give an uncertainty of $\pm 0.2\%$. Assuming the worst possible conditions, namely, that all errors are additive, we arrive at a maximum error of $\pm$ 0.4% in the estimation of α.

RESULTS AND CONCLUSIONS

Four analyses were carried out at each of the temperatures 6.0°, 12.0°, 18.0°, and 24.0°C (Table 1). It may be seen from Figure 2 that the absorption coefficients found for oxygen in distilled water show good agreement with the classical data of Winkler and Fox, lying essentially between their values. We are unable to reproduce the substantially lower revised values.

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Larvae and Pupae of the Species of *Cryptochironomus* (Diptera) in Michigan[1]

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ABSTRACT

Collections from several diversified habitats in Michigan yielded larvae belonging to the genus *Cryptochironomus*. Subsequent collections were made to obtain the immature forms for laboratory cultures from which the adult midge was obtained. The reared adults were then associated with their respective immature forms. Information obtained from the associated specimens and museum specimens of other investigators formed the basis of a key for the Michigan species. Thus, the larval and pupal stages for *C. sorex*, *C. fulvus*, *C. blarina*, *C. digitatus*, and one new provisional species are given. In addition to the larval and pupal morphology, other pertinent facts regarding the biology of the species are given when known.

INTRODUCTION

The genus *Cryptochironomus* constitutes a small group in the midge family Tendipedidae (= Chironomidae). Only six species are recognized in the genus under the proposed change in family name. Since Johannsen's work in 1937 very little information has been published that would be of value in associating the immature stages with the adult form. For this reason it has been difficult to determine the immature forms of the genus. It is the purpose of this paper to clarify the status of *Cryptochironomus* which were found in Michigan during the investigation. The genus is here interpreted essentially as defined for adults by Townes (1945).

HISTORY

Johannsen (1905, 1937), Malloch (1915), Muttkowski (1918), and Richardson (1921, 1925, 1928) have recorded pertinent taxonomic and ecological data for the genus in the United States. Several major taxonomic revisions and ecological data have appeared in European literature since Meigen (1800, 1803) first published on the genus *Tendipes* (= *Chironomus*). The revisions in taxonomy (Harnisch 1923, Edwards 1929, Goetghebuer 1937, and Lenz 1941) were the results of additional descriptions of a new species. A complicated system of nomenclature arose as a result of the revisions. A summary of all known European species of *Cryptochironomus* as compared to the American species is given in Table 1.

In the United States Johannsen (1937) based his nomenclature of *Cryptochironomus* in part upon Edwards (1929). In 1945 Townes raised the group *Cryptochironomus* to the rank of genus. This revision was based upon the original designation by Kieffer (1918). As a result of the revision the subgenera *Harnischia* (*Harnischia*) and *Harnischia* (*Cladopelma*) were separated from the genus *Cryptochironomus* to distinct groups. However, Hennig (1950) continued to recognize 12 subgenera of immature stages and 4 groups of adults under the genus.

The combining of the genera *Harnischia* and *Cryptochironomus* is apparent after a study of the characteristics used by Townes (1945) to differentiate the adults. The following is a summary of the characteris-

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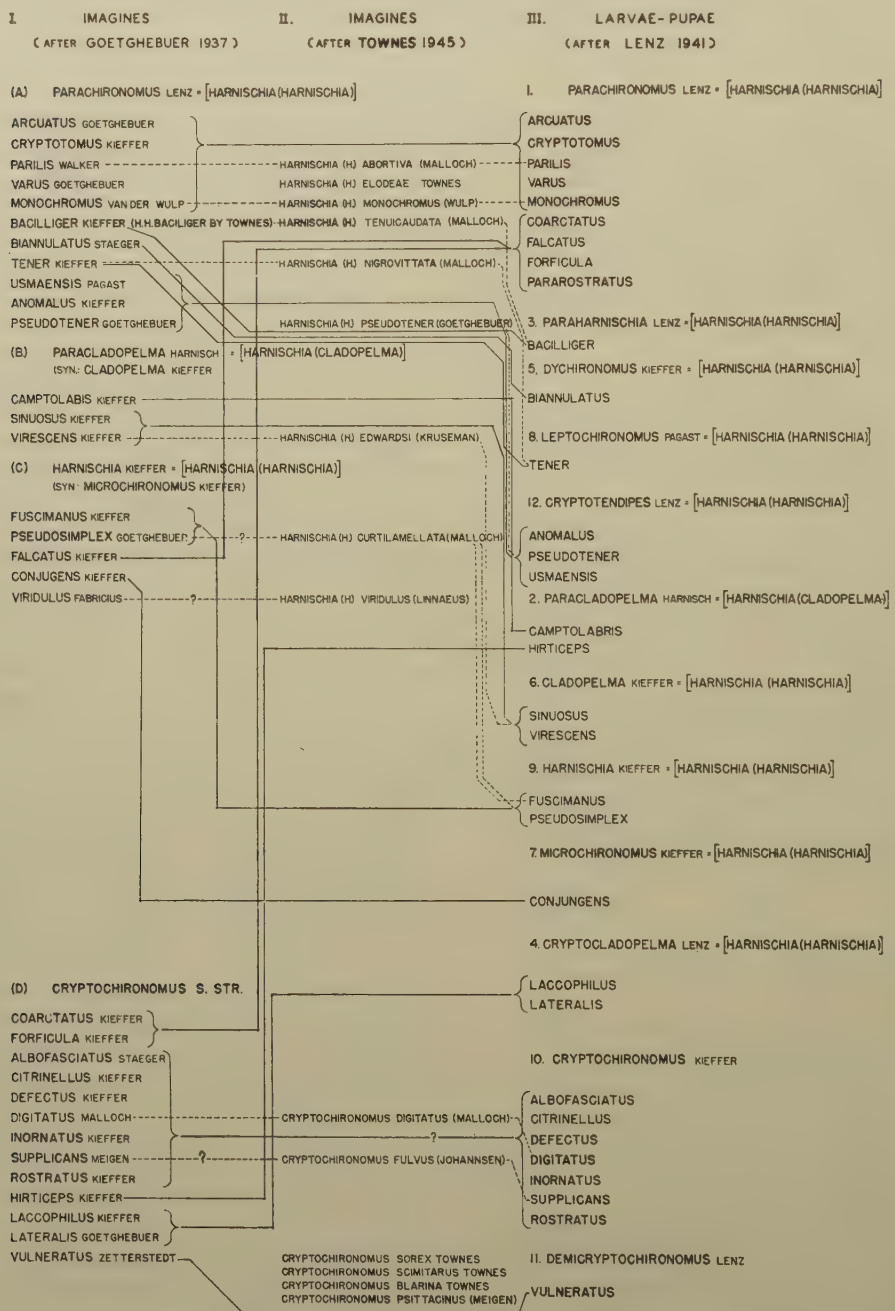
TABLE 1. *Comparison of nomenclature by Goetghebuer, Townes, and Lenz for European and North American species of* Cryptochironomus

Broken lines represent morphological differences between "larval-pupal" and adult systematics drawn from quantitative morphological statistics. Solid lines designate characters of primary importance. (From Hennig 1950, Part II, p. 287. Chart slightly modified.)



tics used in their identification. Adults of both genera have an anal point present, the style and coxite anklosed, and a very small, inferior appendage. In the genus *Harnischia* this appendage, if present at all, is a small membranous lobe without microtrichia, while in the genus *Cryptochironomus* the lobe is sclerotized. Townes (1945) uses the presence of one or more apical bristles on the inferior appendage to separate the two genera.

ECOLOGY

In Michigan *Cryptochironomus* larvae have been found at depths of from several inches to over 10 feet (Table 2). Some species illustrate a wide choice of hydrosol types ranging from silt, marl, and shells to mixtures of detritus and silt and fibrous peat. In some areas the vegetation consisted of *Ceratophyllum* sp., *Vallisneria* sp., and *Potamogeton* sp. Wesenburg-Lund (1943) reported that larvae were frequently found inhabiting a thin layer of mud covering level sand areas of beach zones. The immature forms also inhabit similar habitats in lenitic waters. The Michigan fauna never has been found to be extremely abundant in any collecting area.

The food habits for larvae of the genus as described in the literature are confused. Muttkowski (1918) found from examining stomachs of several hundred larvae of *C. digitatus* and several other species from Lake Mendota that the food is composed of large quantities of organic matter and diatoms. The food of *C. digitatus* larvae appears to be composed of organic ditritus, diatoms, algal spores, and small tendipedid larvae. Larvae of this species are consumed, in turn, by hydrachnids, *Hyalella azteca*, darters, and *Cottus* sp.

Larvae of *C. psittacinus* have also been reported to be carnivorous. Lindeman (1941) reported that this species (= *Chironomus stylifera* Johannsen) represented a "carnivorous larval group" of an otherwise herbivorous genus. He considered this to be the most important creeping predator in the central portion of the lake. However, Wesenburg-Lund (1943) concluded that all the larvae of *Cryptochironomus* are carnivorous, based on their greater activity, keener sense organs, heavy labral bristles, and formidable mandibles. Because of the carnivorous habits, the larval forms are believed to display a minor form of territoriality. Therefore, dense larval populations of this midge would not be expected to be found in aquatic habitats. Wesenburg-Lund also reported that *Cryptochironomus* larvae were preyed upon by small schools of carp, which were caught in turn by diving birds.

ADULTS

The adults of this genus are in general moderately small. Adults of *C. fulvus* are about 2 mm long, whereas those of *C. psittacinus* range from 4–6 mm in length.

Palpus with 4 segments; male flagellum with 11, female with 5 segments; antennal ratio 2.7 to 5.5; frontal tubercles present or absent; pronotum evenly and moderatedly narrowed towards the center, not produced or notched medially, even with or slightly surpassed by the anterior part of the mesoscutum; squamal fringe present; wing membrane without macrotrichia; fork of *Cu* somewhat beyond *r-m*; ends of R_1 and R_{2+3} slightly but distinctly separated; ends of R_{4+5} and M equidistant from wing apex; leg ratio 1.1 to 2.1; tarsal beard present or absent; fore tibia with an inner apical low rounded scale which projects only slightly beyond a similar scale on the other side of the tibia; combs of middle and hind tibia broadly triangular, similar in shape, adjacent but usually with a distinct notch between them, each with a short spine; pulvilli conspicious, lobes entire.

Male genitalia: anal point, superior appendage small, lobed-shaped, and with many setae; inferior appendage very small, sclerotized, and with several setae; style and coxite anklosed (Townes 1945).

The genus is widely distributed in the northern part of the United States. Field collections of adults indicate a range from California to New York, Massachusetts, and Maryland. Adults have been collected at altitudes of 5,000 feet on Mt. Ranier. In Michigan adults have been collected from Isle Royal to Van Buren and Washtenaw Counties in the Lower Peninsula.

A complete seasonal distribution for the several species is unknown. In Michigan *C. fulvus* adults appear from early spring to fall. *C. digitatus* and *C. psittacinus* adults

TABLE 2. *Ecological preference of the larvae of Cryptochironomus* sens. str.

Species	Depth	Bottom deposit	Vegetation	Location
C. fulvus	8–10 in.	Silt and fine clay, edge of current	*Ceratophyllum* sp. *Vallisneria* sp. *Potamogeton* sp. Water lilies	Millpond, Mt. Pleasant, Isabella Co., Mich.
	15–30 ft.	Silt, marl, shells	None	Coldwater Lake, Isabella Co., Mich.
	No data	Littoral, sublittoral	No data	Muttkowski (1918)
C. blarina	16 in.	Pulpy peat and sand	*Ceratophyllum* sp. *Potamogeton* sp. Water lilies	Millpond, Mt. Pleasant, Isabella Co., Mich.
	15–30 ft.	Silt, marl, shells	None	Coldwater Lake, Isabella Co., Mich.
C. psittacinus	39 in.	Benthic ooze	No data	Cedar Bog Lake, Minn. (Lindeman 1941)
	0–3 ft.	Mud	None?	Lindeman (1941) as creeping predator on bottom
C. Sp. *A*	8 in.	Surface particulate marl, base large marl particules	Fragments of *Chara* sp. and sedges.	Wycamp Lake, Emmet Co., Mich.
	Specimen taken from brook trout stomach. Trout was 7.3 in. long, wt, 65 g.			Section A, Pigeon River, Otsego Co., Mich.
C. digitatus	36 in.	Shoal, out of current, silt and fibrous peat	*Elodea* sp. *Myriophyllum* sp.	Millpond, Mt. Pleasant, Isabella Co., Mich.
	6–12 in.	Silt and sand	None	Hunt Creek, Cedar Swamp (Sec. C), Mountmorency Co., Mich.
	12–24 in.	Silt, sand, detritus	None	Pigeon River, Sec. B., Otsego Co., Mich.
	No data	No data	No data	Stoner Lake, Alger, Delta Co., Mich.
	15–20 ft.	Silt, marl, shells	None	Coldwater Lake, Isabella Co., Mich.
	No data	Mud Sub-pollutional, 1913–1915 Late-pollutional, 1920–on	No data	Spring Bay (Richardson 1928)
	8 in–20 ft. littoral form	Ammophilous. Sand to fine gravel. Larva forms tubes.	*Myriophyllum* sp. *Chara* sp. *Lemna* sp. *Potamogeton* sp. to None.	Lake Mendota, Wis. (Muttkowski, 1918)
	7–20 ft.	Rock bottom	No data	Muttkowski (1918)
	Shallow	Mild rapids in creeks, rivers, and falls	No data	Muttkowski (1918)
		Rocky reefs in lake	No data	Muttkowski (1918)
	4–6 in.	Common in cobble and gravel	No data	Muttkowski (1918)
		Barrens of plant marl	No data	Muttkowski (1918)
	Opt. depth 0–16 ft.	Opt. habitat sand	No data	Muttkowski (1918)
	No data	Mud	None	Illinois River, below Havana (Richardson 1921)
	No data	Mud	None	Lower Peoria Lake (Richardson 1925)
	Not over 13 ft.	Mud	None	Middle Peoria Lake near Mossville (Richardson 1925)

have been found from April 15 to September 15. Guyer (1956) estimated *C. digitatus* to have but one emergence per year during the last part of May and the first two weeks of June in the vicinity of Lake City, Missaukee County.

METHODS

The adults were reared from larvae collected in the field as previously described (Curry 1956).

Slides were made of the adult genitalia and cast exuviae of the larva and pupa. The material was dehydrated in absolute alcohol, run through xylol, and mounted in balsam. All parts employed in a morphological study were set as flat as possible for detailed study. Photographs were taken and camera lucida drawings made of the morphological structures of the immature stages. Details were drawn in by observation under the microscope. The adults were pinned on minuten pins.

For larvae and pupae preserved material or material freshly killed is best for identification. Preservation has proven satisfactory in 10% formaldehyde with a trace of glycerine added to keep the specimens soft. A specimen prepared for identification must be mounted ventral side up under the coverglass. This may be done in a water mount. Some difficulty may be had with the larger robust specimens. In this case the head is cut off, or mounted under the edge of the coverglass. A slight pressure on the coverglass will spread the mandibles so that they can be viewed better. The mandibles and labial plate should be visible to the investigator. The specimen is then viewed under high power.

MORPHOLOGY OF LARVAE AND PUPAE

The immature forms of the genus *Cryptochironomus* occurring in Michigan have been found to differ greatly from other larval forms of Tendipedini, which is in agreement with the findings of Johannsen (1937). The larvae are recognized by a broad colorless medial tooth and bright red, fusiform body. However, specific color variations were observed for the species *C. digitatus*. Muttkowski (1918) found scarlet larvae to be most common, white or pale green larvae to be next frequent, and a dark red form with green dorsal markings on each segment to be quite rare. Breeding of the three types has proved their identity, but no racial distinctions could be found. The above variations in color may occur in the same locality. Larval specimens from Michigan have small oval-shaped heads. When disturbed, they swim with a violent S-shaped motion.

Keys for the larvae are based upon the morphology of the labial plate and mandibles. The epipharyngeal plate (Fig. 1) is not used in the key due to the great similarity of this structure between species. Furthermore, the larvae do not possess ventral blood gills on the penultimate body segment. Larvae of Michigan species do not appear to exceed 15 mm in length. A comparison of the morphology of the Michigan fauna is found in Table 3.

The pupal exuviae of the Michigan fauna do not have spurs or combs on the lateroposterior angle of segment eight. The respiratory filaments are paired and threadlike. Cephalic tubercles are present, but vary in length and shape. A series of hooks is present on the posterior margin of the second tergite. In addition, spines are present on the posterior borders of many tergites. A comparison of the pupae of several Michigan species is found in Table 4.

KEY FOR LARVAE

1a. Labial plate with 7 long, sharp lateral teeth; medial tooth ⅓ wider than high; lateral teeth all dark, and the plate curved (Fig. 26); apical tooth of mandible 2.8 times as long as wide; lateral teeth project obliquely from mandibles, long and pointed (Fig. 24); premandibles with 3 large teeth (Fig. 25) *Cryptochironomus* sp. *A*

1b. Labial plate with 5 lateral teeth 2a

2a. Lateral teeth of mandible pyramiform; apical tooth of mandible dark (Fig. 10); labial plate with lateral teeth dark; outermost layer tooth distinctly lighter (Fig. 9) *Cryptochironomus fulvus* (Johannsen)

2b. Mandibles and labial plate differ 3a

3a. Mandible with a large black, lunular apical tooth with lateral teeth blunt and dark (Fig. 15); lateral teeth of labial plate all dark, the outermost smallest; medial tooth with distinct spines near the center (Fig. 14) *Cryptochironomus blarina* Townes

TABLE 3. *Measurements (in mm) of some of the anatomical structures of larvae of species of* Cryptochironomus

Structures	*C. sorex*	*C. fulvus*	*C. blarina*	*C. digitatus*	Sp. *A*
Head:					
Length	0.36–0.46	—	0.49	0.14	0.35–0.41
Width	0.25–0.36		0.43	0.11	0.26–0.28
Eye spot	—	—	Two eye spots oval 0.03 reniform 0.03	—	Two eye spots oval 0.01–0.03 oblong 0.02 Single eye spot 0.03 x 0.02
Antennal ratio:	35:20:17:4:2	38:20:15:3:2	35:20:?:?:?	45:20:14:2:3	35:10:10:8:3
Base (width)	0.02	0.02	0.02	0.02	0.02
Ring organ (diameter)	0.01 upper ⅓	0.01 upper ⅓	0.01 upper ⅓	upper ⅓	0.008 upper ⅓
Blade (length)	0.03	0.05	—	—	0.03
Mandibles	0.1	0.14 x 0.05	0.18 x 0.07	0.26 x 0.07	0.11 x 0.05
Premandibles	—	0.11 x 0.04	0.13 x 0.06	0.13	0.08 x 0.03
Labial plate:					
Total length	0.1	0.14	0.14	0.13–0.26 (flat)	0.09–0.12
Medial tooth (width and height)	0.04 x 0.02	0.03 x 0.04	0.05 x 0.03	—	0.03 x 0.02
Segmented styles (length)	—	—	0.04	—	0.03
Maxillary palps (length)	0.04	0.04 x 0.02	0.06 x 0.02	0.06 x 0.02	0.07 x 0.02
Paralabial plate	0.12 x 0.05	0.17 x 0.04	0.16 x 0.05	0.22 x 0.07	0.17 x 0.04
Anal papillae:					
Width	—	—	0.02	0.04 x 0.03	0.03–0.05 x 0.01–0.03
No. filaments	—	—	8	—	8
Anal blood gills	—	—	—	0.22 x 0.08	0.22 x 0.09

TABLE 4. *Measurements (in mm) of some of the anatomical structures of pupae of some Michigan species of* Cryptochironomus

Structures	*C. sorex*	*C. fulvus*	*C. blarina*	*C. digitatus*
Cephalic tubercles	0.1 x 0.02	0.5 x 0.33	0.16 x 0.07	0.2 x 0.06
bristle	one; 0.01–0.02 from tip	one; 0.03 from tip	None observed	None observed
Second tergite				
Medial interruption in row	0.1–0.15	0.09	0.23	0.06–0.16
Hook height	0.08	0.02	0.02	0.02–0.03
Fourth tergites spines: (w x h)				
Large	0.08 x 0.05	0.01 x 0.006	0.01 x 0.004	0.04 x 0.04
Small	0.02 x 0.01–0.05	0.004 x 0.004	0.004 x 0.018	0.004 x 0.006
Seventh tergite spines:				
Large	0.05 x 0.02	omitted	omitted	0.01 x 0.03
Small	0.02 x 0.03			0.01 x 0.01
Eighth tergite spines:				
Large	0.05 x 0.07	0.04 x 0.01	0.01 x 0.02	None present
Small	—	0.004 x 0.008	0.018 x 0.006	
No. anal fin filaments	51–69	62–63	74–77	81–94
Puparium length	5.5	7.0	7.0–8.0	8.5–9.0

3b. Apical tooth of mandible 2.5–2.7 times as long as wide; outer tooth of labial plate as tall as, or taller than, inner teeth; no spines on medial tooth[2] (Fig. 2).....4a

4a. Mandibles with apical tooth 2.7 times as long as wide; lateral teeth peg-like; colorless accessory tooth spine-like (Fig. 3); medial tooth of labial plate low, broadly rounded and colorless; lateral teeth dark, the last without a notch and distinctly projecting above its neighbors (Fig. 2)
Cryptochironomus sorex Townes

4b. Mandibles with apical tooth 2.5 times as long as wide; lateral teeth dark; cusp oblique, or obtuse and angulate; accessory tooth spatulate (Fig. 20); lateral teeth of labial plate dark, outermost tooth distinctly above its neighbors (Fig. 19)
Cryptochironomus digitatus (Malloch)

KEY FOR PUPAE

The pupae of provisional species *A* were not observed.

1a. Cephalic tubercles bituberculate (Fig. 12a); the middle pair elongate, the outer shorter; a single seta on inner margin near apex of inner pair
Cryptochironomus fulvus (Johannsen)

1b. Cephalic tubercles unbranched.....2a

2a. Cephalic tubercle short, conical, and without a seta near tip.....3a

2b. Cephalic tubercles short (0.1 mm) and pointed; a bristle 0.01–0.02 mm from the tip of tubercle (Fig. 5); puparium about 5.5 mm long; anal fin with 51–69 filaments
Cryptochironomus sorex Townes

3a. Cephalic tubercle short (0.16 mm) and pointed (Fig. 17a); cuticle of abdominal segments not reticulate; about 75 filaments in anal fin
Cryptochironomus blarina Townes

3b. Cephalic tubercles elongate (0.02 mm) and pointed (Fig. 22a); cuticle of abdominal segments reticulate; anal fin with 81–94 filaments
Cryptochironomus digitatus (Malloch)

DESCRIPTIONS OF THE SPECIES

Cryptochironomus sorex Townes (1945)

Larva. Three slides of complete association were obtained from Dr. H. K. Townes. The larval head is slightly longer than wide, ochreous, with darkened mandibles and labial plate. The labial plate (Fig. 2) is 0.1 mm wide, the medial tooth colorless, twice as long as wide, pigmented laterally, with a small notch visible at the point of origin of the pigment; lateral teeth dark brown, sharply pointed, the outermost pair appear to be fused at their bases; no notch in the outermost tooth which in some specimens appears lighter than its neighbors and projects distinctly higher than the medial tooth; the labial plate is curved. The mandible (Fig. 3) is 0.1 mm long, the apical tooth dark brown and 2.7 times as long as wide; the lateral teeth long, dark and peg-shaped; the accessory tooth colorless, narrow and pointed; the body of the mandible ochreous with 2 setae on the dorso-lateral margin and a simple ventral brush. The premandibles could not be observed from the specimens. The segments of the antennae (Fig. 4) bear a ratio of 35:20:17:4:2 to one another; the ring organ is located in the upper ⅓ of the basal segment; the blade is 0.03 mm long. A pair of segmented styles is present on the labrum. The maxillary palps are armed with about 5 spines. The anal papillae have about 8 bristles. Table 3 has measurements of some of the anatomical structures for the species.

Pupa. The pupal exuviae were 5.5 mm long, the cuticle of the thorax brownish, abdominal segments ochreous; the thorax smooth except for small areas of minute nodules around the anterior portion. The cephalic tubercles (Fig. 5) are small, cone-shaped, and bear a bristle near the tip. One specimen (Fig. 6) had tubercles much shorter but wider at the base. The respiratory organs are paired, with numerous thread-like filaments. The surface of the abdominal segments 1–6 is smooth except for a reticulate pattern along the posterior border and shagreen in the center; segments 4 to 8 bear a few scattered bristles; a row of minute hooks is located along the posterior border of the second tergite (Fig. 7a), which is interrupted for a distance of 0.1 to 0.15 mm on the medial line; a row of spines is

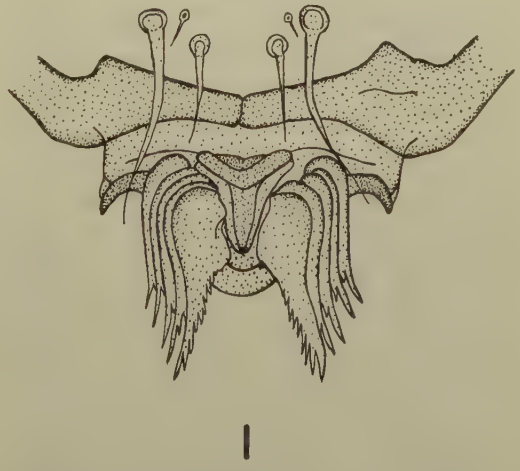


FIG. 1. Epipharyngeal area of *Cryptochironomus digitatus* (Johannsen).

[2] The hypopharynx which can be seen through the colorless median tooth might be interpreted as spines.

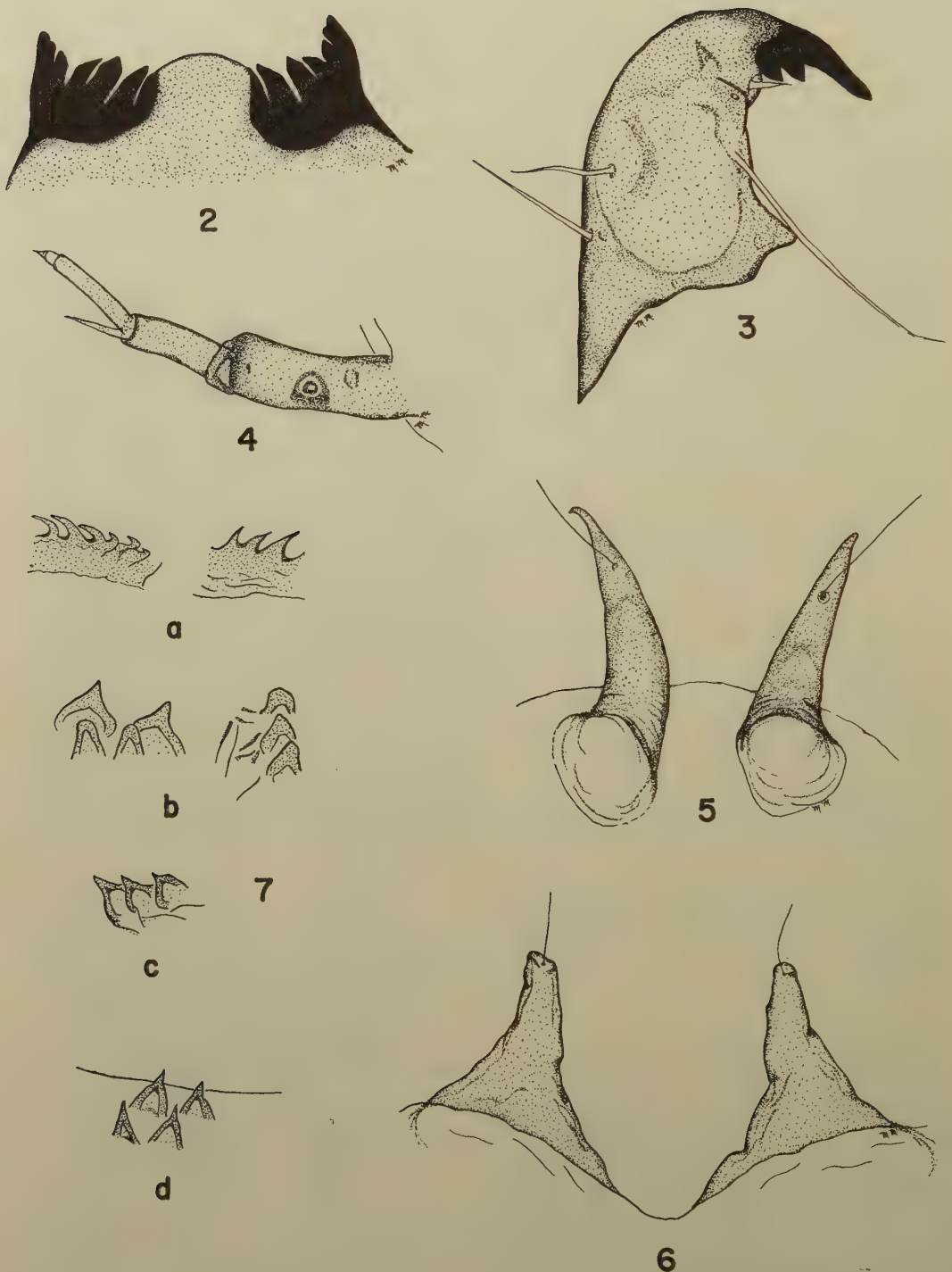


FIG. 2–7. *Cryptochironomus sorex* Townes: 2. labial plate; 3. mandible; 4. antenna; 5. cephalic tubercles; 6. cephalic tubercles of variant pupa; 7. armature on abdominal segments of pupa: *a*. hooks on 2nd segment, *b*. spines on 4th segment to show variation, *c*. spines on 7th segment, *d*. spines on 8th segment.

located along the posterior border of tergites 4–7; segments 4, 7, 8 (Fig. 7b, c, d) have stronger spines on the median line; no comb or spur is present on segment 8; the anal fin is composed of 51–69 filaments and terminates in two small conical proeesses.

Adult. Head and thorax ochreous, abdomen greenish with the apical part more or less brown; flagellum (except basal segment) and palpus brown; legs ochreous; fore tibia and tarsus and apex of fore femur brown; apical part of middle and hind tarsi brown (Townes 1945). Townes considers the long, broad anal point, and narrower converging scleromes as distinctive.

Distribution. New York.

Seasonal distribution. Adults have been collected during May, June, August, and September.

Cryptochironomus fulvus (Johannsen 1905)

Synonomy: *Chironomus fulvus* (Johannsen 1905, Malloch 1915, Townes 1937, 1938)
Chironomus abbreviatus (Malloch 1915, Muttkowski 1918)
Chironomus parvilamellatus (Malloch 1915)
Chironomus sp. *C.* (Malloch 1915)
Chironomus Mallochi (Kieffer 1919)
Chironomus (*Cryptochironomus*) *fulvus* (Johannsen 1937)
Chironomus (*Cryptochironomus*) *parvilamellatus* (Beyer 1941)

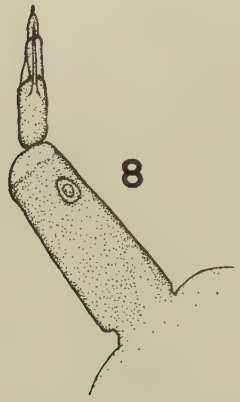
Larva. Malloch (1915) and Johannsen (1937) have described the larva briefly. Johannsen (1937) stated that the larvae and pupae of *C. fulvus* did not appear to differ in structural character from those of *C. digitatus* (= *C. stylifera* var. *a*). In this study association of the respective adults with their immature forms has revealed structural differences in both the larval and pupal stages. The larva of *C. fulvus* is blood red and fusiform; the head ovate and of a uniform color except for the gular plate which darkens toward the labial plate. The anterior pseudopods with weak apical hairs, the posterior pseudopods with normal apical claws. The anal papillae are inconspicuous and each bears about 6 hairs. The dorsal blood gills are large and 2.5 times as long as wide. This description does not fully agree with Malloch (1915) or Johannsen (1937). The antennae (Fig. 8) are 0.13 mm long, with the segments bearing a ratio of 38:20:15:3:2 to one another; the ring organ has a diameter of 0.01 mm and is in the distal third of the basal segment. The labial plate (Fig. 9) has five dark lateral teeth, the innermost long and pointed, the outermost blunt, lighter, and projecting slightly above its neighbors; the colorless medial tooth has a smooth margin and is ⅓ as wide as the labial plate; the pigmented crescent has its origin at a slight notch on the margin of the tooth. The maxillary palps are 0.04 mm long and 0.02 mm wide at the base. The paralabial plates are 4 times as long as wide, medially oblanceolate and decurved laterally, distinctly striate, pointed medially, and are separated on the medial line. The lateral teeth of the mandible are pyramiform and darkened; the apical tooth dark (Fig. 10); the accessory tooth is spatulate and colorless; the brush on the specimen was composed of a single bristle, and a preapical comb was absent. The premandibles (Fig. 11) have six teeth, the 3 distal teeth are largest, the innermost the smallest and conjoined to its neighbor. The epipharyngeal area has about 4 blades and several scales on each side, being similar to *C. digitatus* (Fig. 1). A bar and comb are not present as in the genus *Tendipes*. The plate is a triangular area with a single spine at its apex and a depressed bar at the base. This plate is difficult to observe without dissection. A triangular plate is also present with a spine projecting conspicuously from the posterior border on the medial line. The edge of the plate is serrate. The base is formed by an elongated bar depressed in the center.

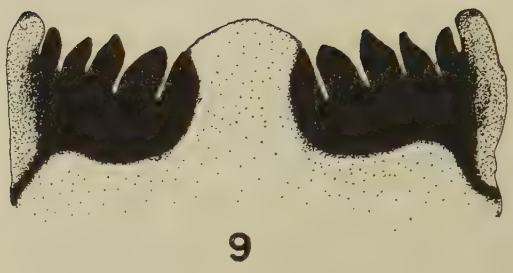
Pupa. The exuvial cuticula examined was 7 mm long, thoracic segments brown, and abdominal segments ochreous. The cuticle of the thorax is smooth except for small areas of minute nodules at the base of the cephalic tubercles. The cephalic tubercles (Fig. 12a) are bituberculate, the medial pair elongate and curved, the outer shorter; a single seta located on the inner side of the medial pair near their apices; the tubercles sharply pointed and blackened at the tips (not shown in Fig. 12a). The respiratory organs are paired and have tufts of numerous thread-like filaments. A large nodule lies near the base of the leg buds and is covered with spinules, some of which are curved. The surface of abdominal segments 1 to 6 smooth with shagreen in the center of each segment; a few scattered pairs of bristles on tergites 2 to 8; a row of minute hooks of various shapes located on the posterior margin of the second tergite (Fig. 12b), the row interrupted by a distance of 0.09 mm on the medial line; tergites 3–8 with a transverse row of spinules on the posterior margin (Fig. 12 c, d); segment 8 without comb or spur. The caudal fin contains about 62 filaments and terminates in two short conical processes.

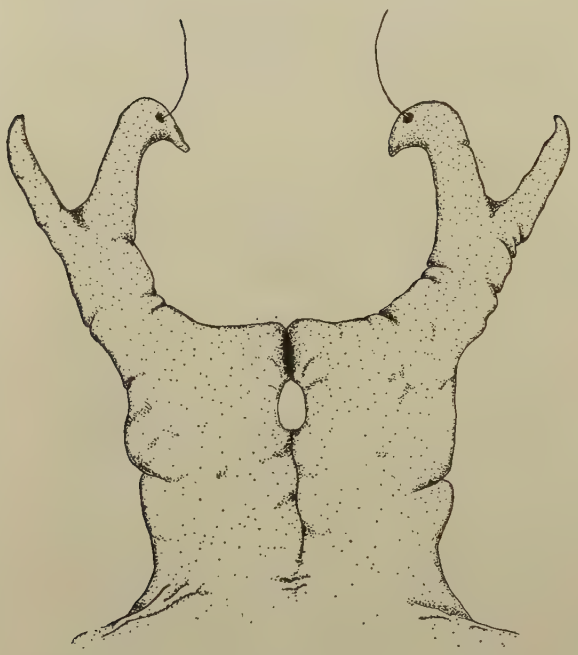
Adult. A small midge; the specimens in the collection vary from 2 to 3 mm long. Head and thorax ochreous, abdomen greenish with apical parts more or less brown; flagellum (except basal segments) and palpus brown; legs ochreous; fore tibia, tarsus, and apex of fore femur brown; apical part of middle and hind tarsi brown (Townes 1945). Preserved specimens in one collection have thorax ochreous, with ferruginous markings; abdomen ferruginous. Frontal tubercles small or absent. Townes (1945) considers the slender anal point and stout, slightly curved style ax distinctive.

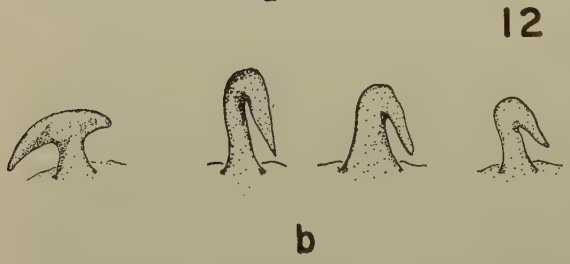
Emergence data. Larvae collected VIII/1/55; adults emerged on VIII/5/55; 4 days required for transformation.

Ecology. Larvae of *C. fulvus* were collected in a bottom deposit of silt and fine clay at a depth of









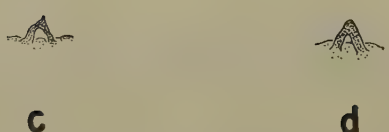


FIG. 8–12. *Cryptochironomus fulvus* (Johannsen): 8. antenna; 9. labial plate; 10. mandible; 11. premandible; 12. cephalic tubercles and armature on abdominal segments of pupa: *a*. cephalic tubercles, *b*. hooks on 2nd segment, *c*. spine on 4th segment, *d*. spine on 8th segment.

8–10 inches near edge of current. The vegetation consisted of pond lilies, *Ceratophyllum* sp., *Vallisneria* sp., *Potamogeton* sp. Larvae have also been identified in samples from depths of 15–30 feet. The bottom deposit at the greater depths consisted of silt, marl, and small snail shells. Townes (1938) found the species living in a shallow eutrophic lake at depths of 0.8–26 feet. The hydrosol was composed of plant detritus and silt, with plants extending only to a depth of 13 feet. This species is considered to be distributed only to the sublittoral by Muttkowski (1918). Adults have been found on Mt. Rainier at an elevation of 5,000 ft. (Townes 1945).

Distribution. *Michigan:* Arenac, Bay, Benzie, Chippewa, Isabella, Isle Royale, Mackinac, Midland, Missaukee, Montcalm, Van Buren, and Tuscola counties. *North America:* California, Colorado, District of Columbia, Florida, Idaho, Illinois, Iowa, Kansas, Louisana, Manitoba, Maryland, Massachusetts, Minnesota, Mississippi, Montana, Nebraska, New Jersey, New York, Oklahoma, Ontario, Quebec, Rhode Island, Virginia, and Washington.

Seasonal distribution. Adults occur throughout the growing season. Early and late collections were made April 22 at Grand Tower, Illinois; May 8, Forest Glen, Maryland; May 15, South Hadley, Massachusetts; September 12, Ithaca, New York; September 16, Davenport, Iowa; October 31, West Palm Beach, Florida.

Cryptochironomus blarina Townes (1945)

Larva. The body is fusiform, blood red in color; there are 2 pairs of anal gills, and the ventral gills absent; the head ovate and uniformly ochreous with the posterior margin of the labial plate darkened near the teeth. The antennal base (Fig. 13) about 2 times as long as wide with the ring organ on the distal ⅓ of the segment; the first segment is 1.7 times as long as the second. Further observations could not be made as the antenna was destroyed in part during dissection. Two eye spots are present, the anterior spot large and reniform and separated by a distance of ⅓ the length of the larger eye spot. The anterior border of the labrum has a pair of segmented styles 0.05 mm long with the apical segments oblique. The paralabial plate is decurved, 3 times as long as wide, striated and separated on the medial line. The labial plate has 5 dark lateral teeth, the innermost the longest, the outermost the shortest and with a distinct notch (Fig. 14). The medial tooth is colorless with two small spines near its center and ⅓ as wide as the labial plate. The dark areas on the tooth have their origin at two distinct notches about ⅕ the distance toward the center, and then are projected vertically down the tooth. The maxillary palps have the usual armature of styles and blades. The mandible (Fig. 15) has a long curved, black apical tooth with the color extending to the two short blunt lateral teeth. The premandibles (Fig. 16) are twice as long as wide, with 6 teeth, the largest located at the distal end, the smallest at the proximal end; the fourth and fifth are conjoined along their inner borders. The epipharyngeal area has the usual serrated blades and scales (Fig. 1).

Pupa. The specimen observed had an exuvia 8 mm long, thorax dark brown, and abdominal segments fulvus; respiratory organs paired with tufts of thread-like filaments; cephalic tubercles (Fig. 17a) short and conical, the cuticle reticulate near their bases and the rest composed of minute nodules. A stalked tubercle covered with spines and hooks was located near the base of the leg buds. The surface of the abdominal segments appears smooth with a few scattered pairs of bristle on sternites 2 to 8; a row of minute hooks (Fig. 17b) on tergites 3 to 8 (Fig. 17c, d) with a transverse row of spinules, the largest on tergite 8 (Fig. 17d). Segment 8 without a comb or spur, and the caudal fin with about 74–77 filaments; the segment terminating in two conical processes.

Adult. Head and thorax light greenish brown, strongly pruinose with brown markings; abdomen dark brown; pedicel, flagellum except base, and palpus brown; legs pale brownish green, fore tibia brownish at base and apex; fore tarsus and apical part of middle and hind tarsus brown (Townes 1945). Townes considers the long, narrow, evenly curved style as distinctive.

Ecology. Larvae of this species were collected July 1, 1955, from a bottom deposit of pulpy peat and sand. Plant associations were *Certophyllum* sp., *Potamogeton* sp., and water lilies. Larvae of this species have been identified from collections taken at depths of 15–30 feet in deposits of marl, silt, and shells.

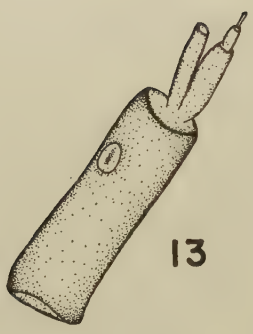
Emergence data. Larvae collected on July 1, 1955, transformed to pupa on July 5, 1955; the adult emerged on July 7, 1955, requiring 48 hours for pupal transformation.

Distribution. Mayville and Farmingdale, New York; Isabella County, Michigan; and Normandale, Ontario.

Cryptochironomus digitatus (Malloch 1915)

Synonomy: *Chironomus digitatus* (Malloch 1915, Muttkowski 1918, Richardson 1921, 1925, 1928)
Chironomus (*Cryptochironomus*) *stylifera* var. *a* (Johannsen 1937)
Chironomus (*Cryptochironomus*) *digitatus* (Johannsen 1937)

Larva. The larvae are blood red, fusiform, and about 10 mm long when full grown. Muttkowski (1918) listed three color distinctions for the larvae: (1) scarlet larvae were the most common, (2) white or pale green the next most frequent, and (3) a least frequent form was colored dark red with green dorsal markings on each segment. These variations may occur side by side in the same habitat. The larva has an oval head of uniform color varying from ochreous to fulvus; the gular portion is darkened along the posterior margin of the sclerite; two distinct eyespots are present separated by a distance of 0.02 mm; a pair of 3-



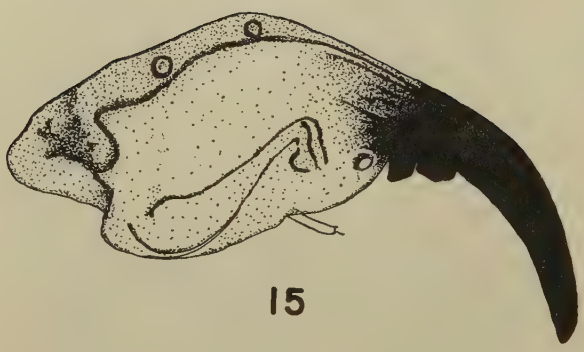


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FIG. 13–17. *Cryptochironomus blarina* Townes: 13. antenna; 14. labial plate; 15. mandible; 16. premandible; 17. cephalic tubercles and armature on abdominal segments of pupa: *a*, cephalic tubercles, *b*. hook on 2nd segment, *c*. spine on 4th segment, *d*. spine on 8th segment.

segmented styles is on the labrum; the antennal segments (Fig. 18) bear a ratio of 45:20:14:2:3 to each other, and the ring organ is located in the distal third of the basal segment. The paralabial plates are obliquely decurved, three times as long as wide and distinctly striate. The labial plate (Fig. 19) has 5 lateral teeth with the outermost tooth distinctly lighter and taller than its neigh-

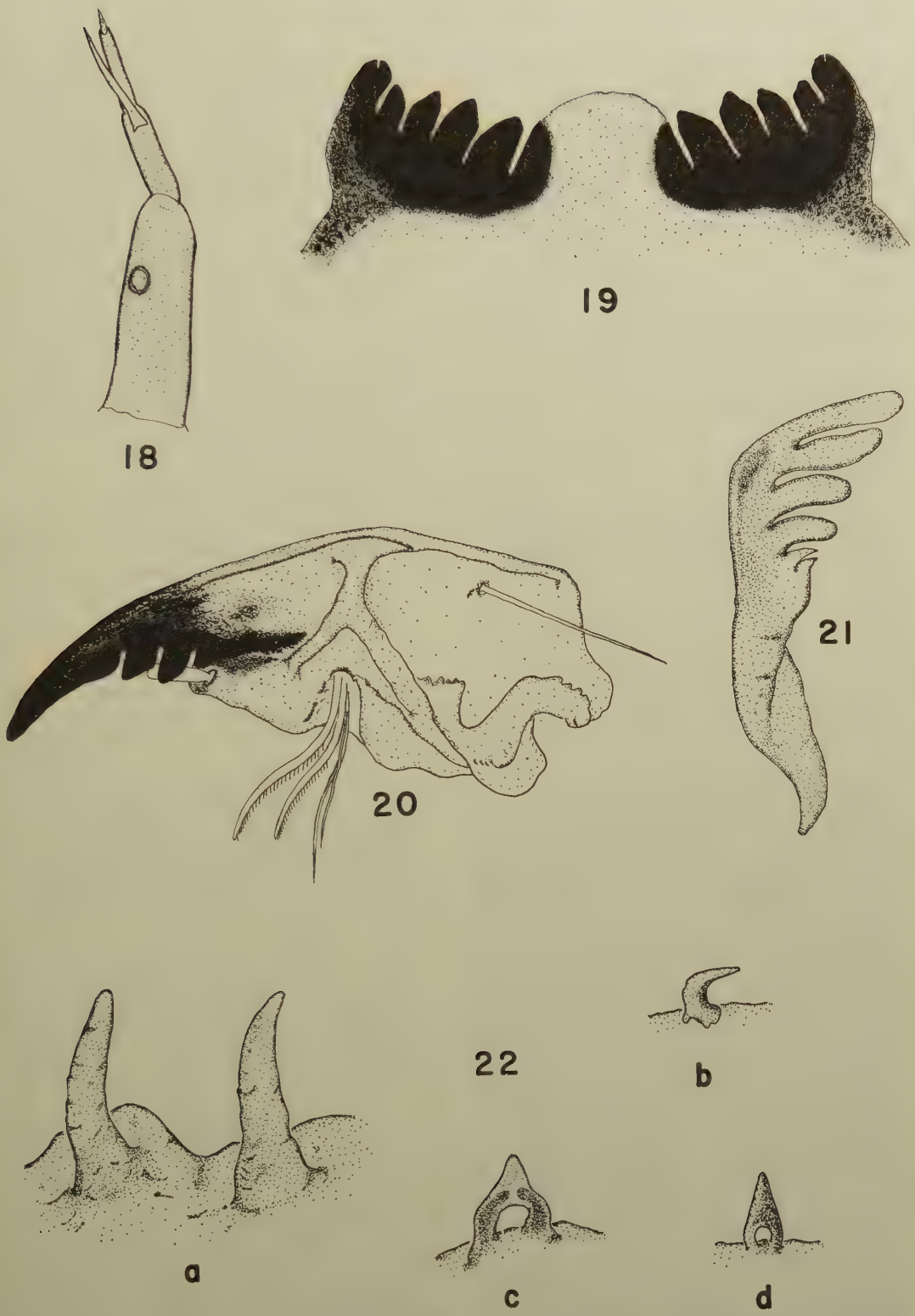

FIG. 18–22. *Cryptochironomus digitatus* (Malloch): 18. antenna; 19. labial plate; 20. mandible; 21. premandible; 22. cephalic tubercles and armature on abdominal segments of pupa: *a*. cephalic tubercles, *b*. hook on 2nd segment, *c*. spine on 4th segment, *d*. spine on 7th segment.

bors and with a distinct notch in the crown; the medial tooth is ⅓ as wide as the labial plate, colorless, with an even margin and a slight notch on each side of the tooth at which point the pigmented area starts and curves downward to the lower portion of the plate. The mandible (Fig. 20) has a relatively short dark apical tooth with dark obtuse annulate lateral teeth, a colorless spatulate accessory tooth, and a brush of two large serrated bristles and two small bristles; there is no preapical comb; two setae are located on the lateral margin of the mandible (only 1 shown in Fig. 20). The premandibles (Fig. 21) are about 3 times as long as wide, of uniform color and widest at the apex; the armature consists of 6 teeth, the largest at the distal end; the last two are small, short, and conjoined. The epipharyngeal area has about 6 blades and several scales. The maxillary palps are 3 times as long as wide with the usual blades and bristles. The larva has two pairs of anal blood gills with the ventral pair larger and bladder-like, the dorsal elongate with several long filaments located directly above in the proleg cuticula; ventral gills absent; anal papilla about as long as wide, with a small filament on the lateral margin, and a pencil of 8 long filaments at the distal end of each papilla.

Pupa. The specimen exuviae 9 mm long with dark brown thorax, and abdominal segments fulvus; the cephalic tubercles (Fig. 22a) elongate, conical, and curved ventrally, and apparently with a preapical bristle; the respiratory filaments paired and thread-like; the cuticle of the thorax with minute nodules which cover a larger area than in either *C. fulvus* or *C. blarina;* abdominal segments 1–5 reticulate; the second segment with a row of hooks (Fig. 22b) separated in the center by a distance of 0.06 mm; tergites 3–7 with a row of spinules along the posterior margin (Fig. 22c, d); the abdominal segments with a few scattered bristles; the anal lobe with a fringe of about 90 filaments terminates in two blunt, darkened conical processes, below which are a pair of lanceolate processes longer than those of *C. blarina.*

Adult. The adult male of *C. digitatus* is larger than that of *C. fulvus* or *C. blarina.* The thorax is ochreous with head and thorax marked by a darker ochreous; flagellum (except base) and palpus brown; legs ochreous; fore tibia brownish at base and apex; fore tarsus and apical part of middle and hind tarsus brown; posterior margin of abdominal tergites 1 through 4 with transverse brown band; genitalia tinged with brown. Some specimens, especially those collected in early spring, with olive-green ground color, with mesoscutal vittae and other darker markings brownish-black. Townes (1945) regards the shape of the style, intermediate between *C. blarina* and *C. psittacinus* as distinctive.

Ecology. Larvae of *C. digitatus* have been collected from the greatest variety of habitats (Table 2). This finding is in agreement with Muttkowski (1918). Depth ranged from 6 inches to 30 feet, generally in shallow water. The optimum depth is 0–5 meters (Muttkowski 1918). Richardson (1928) found this species in Spring Bay (1913–1915) and listed it as *late sub-pollutional,* but in 1920–25 the species was listed as *early pollutional* to *late pollutional.* The U. S. Public Health Center, Cincinnati, Ohio, does not consider the species as an indicator of pollution, but an organism living "in cleaner" areas (MacKenthun pers. corres.). Muttkowski (1918) collected larva of *C. digitatus* from Lake Mendota, a typical eutrophic lake, where the species was found to be widely distributed in the sandy littoral. Larvae have been found on rock bottom to the depth of 2–6 meters in the rapids of creeks, rivers, and falls as well as on rocky reefs in Lake Mendota. This is not in full agreement with Thienemann (1954). Larvae have also been found in gravel, mud, and plant-inhabited bottom deposits. Larvae of this species have been reported (Richardson 1921, 1925) in mud at depths not over 13 feet in the Illinois River below Havana in Lower and Middle Peoria Lake.

Emergence data. A larva collected on June 1, 1955, completed pupal transformation on June 10, and the adult emerged June 12. A second larva of this collection transformed to the pupal stage on July 7, and had transformed to an adult by July 8. A third larva collected on June 27, 1955, completed adult emergence on July 1, 1955.

Food. Diatoms and detritus observed in several mounted specimens. Diatoms, Protozoa, algal spores, smaller tendipedid larvae.

Predators. Shore fishes, especially darters and *Cottus,* hydrachnids, and *Hyalella azteca.* The latter has been observed eating the pupae.

Distribution. *North America:* California, Illinois, Iowa, Massachusetts, Michigan, Minnesota, New Jersey, New York, Ohio, South Dakota, and Ontario. *Europe:* Lake Leman, Switzerland (Zabrowska, in Malloch 1915).

Seasonal distribution. Adults collected from April 30 at Davenport, Iowa, to May 8 at Syracuse, New York, to September 13 at Trenton, Ontario.

Cryptochironomus species A

Larva. Only two larval specimens of this species were collected. One was removed from the stomach of a 7.3-inch brook trout, June 23, 1953; the other was collected from a particulate marl bottom at Wycamp Lake, Emmet County, Michigan, in 1955. The larvae were 6–7 mm long; mounted specimens have a yellow body color with a reddish tinge; the head is longer than wide, of a uniform ochraceous color except for the dark brown labial plate; two distinct eye spots are present; the antenna (Fig. 23) is 0.122 mm long, and the segments bear the ratio of 35:10:10:8:3 to one another; the ring organ is in the distal third of the basal segment; the blade is 0.03 mm long; the mandibles (Fig. 24) have a short, pointed apical tooth, with dark oblique, pyriform lateral teeth similar to *C. fulvus*; the premandibles (Fig. 25) are twice as long as wide with only three teeth visible, the two distal ones

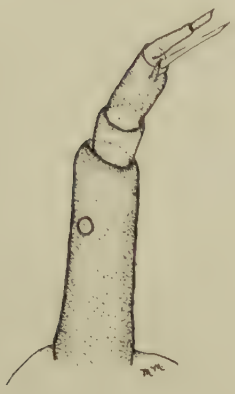

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FIG. 23–26. *Cryptochironomus* species *a*: 23. antenna; 24. mandible; 25. premandible; 26. labial plate.

being the smallest; the labial plate (Fig. 26) has the usual colorless medial tooth, with seven long, pointed dark teeth of uniform height; the paralabials are distinctly striate; the epipharyngeal plate is similar to *C. digitatus* (Fig. 1), and 2 segmented styles 0.03 mm long on the labrum; anal blood gills 2½ times as long as wide and large; the anal papillae longer than wide, and the pencil of hairs consists of 8 filaments. The species was not reared.

Distribution. Wycamp Lake, Emmet County, and Pigeon River, Otsego County, Michigan.

Seasonal distribution. Larvae collected in June and July.

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Phytoplankton Nitrogen in the Oceans[1]

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ABSTRACT

The ratio of cellular nitrogen and chlorophyll has been studied in cultures of marine phytoplankton during and after recovery from nitrogen deficiency. The time factors were observed to vary together; however, the ratio was only constant when the cells were sufficient in nitrogen. Since nitrogen deficiency produced chlorotic cells in all species studied, the degree of deficiency could be ascertained by measuring the ratio of carotenoid to chlorophyll *a*. Thus an expression utilizing the ratio of plant pigments has been formulated to compute cellular nitrogen from chlorophyll *a*. This pigment:nitrogen relationship has been applied to estimate the quantity of nitrogen in natural populations of phytoplankton.

The amounts of nitrogen in phytoplankton estimated from plant pigment measurements has been observed to vary between 18–70% of the total particulate matter.

INTRODUCTION

In the surface waters of the oceans particulate nitrogen occurs in planktonic plants, animals, bacteria, and detritus. The contribution of any one of these categories to the total particulate nitrogen is difficult to evaluate because of non-uniformity as to shape, numbers, and particle-size distribution. One promising approach, however, toward indirect estimates of the amount of organic nitrogen in phytoplankton is the use of a nitrogen:chlorophyll equivalent. That nitrogen and chlorophyll are closely related in plant material has been observed by numerous workers. Noack (1925, 1926), Pirson (1937), Fleischer (1935), and Harvey (1953) reduced the chlorophyll content of plants by rendering them deficient in nitrogen. Michael (1935) found leaves high in chlorophyll also high in protein; Boersch (1932) and Kingsbury (1956) have observed changes in the proportions of chlorophyll and carotenoid in nitrogen-deficient cultures in blue-green algae.

Since the ratio of cellular nitrogen:chlorophyll in the phytoplankton cannot be evaluated in nature, we have used laboratory cultures of marine phytoplankton for this purpose. The changes in chlorophyll *a* and cellular nitrogen were determined in a culture of *Phaeodactylum tricornutum* (Hendy) during and after recovery from nitrogen deficiency. Under these conditions the nitrogen:chlorophyll ratio is not a constant, but varies in a way closely correlated with the degree of chlorosis of the cell which can be represented by a carotenoid:chlorophyll ratio.

The application of the N:Chl[a] ratios, derived from nitrogen-deficient culture experiments, to natural plant communities makes the assumption that similar ratios would obtain for a variety of species under similar physiological conditions. Pigments and cellular nitrogen, measured simultaneously in other species of diatoms and flagellates, tend to confirm this assumption.

METHODS

Nitrate-nitrogen was determined colorimetrically in a Beckman DU spectrophotometer according to the hydrazine sulfate method proposed by Mullen and Riley (1955). Cell material was removed from suspension by flocculation and centrifuging by the von Brand (1935) method. The washed cells were then subjected to a Kjeldahl digestion and the associated nitrogen measured colorimetrically as ammonia according to Riley (1953).

Chlorophyll *a* and carotenoid were measured spectrophotometrically in acetone ex-

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tractions by the Richards with Thompson (1952) method, as modified for use with the millipore filter by Creitz and Richards (1955). The computations for chlorophyll *a* and carotenoid pigments were made from nomographs prepared by Duxbury and Yentsch (1956). Cell numbers were estimated in a Levy Hemocytometer under a 10 x objective.

NITROGEN DEFICIENCY AND CHLOROSIS

A unialgal culture in the logarithmic stage of growth was harvested by centrifuging. The cells were thoroughly washed in nitrate-free millipore-filtered seawater to assure complete removal of any associated available nitrogen. The washed cells were then resuspended in a nitrogen-free medium of the following composition:

Na_2HPO_4	.0002	g
Na_2SiO_3	.005	g
$Na_2S_2O_3$	.0012	g
Ferric citrate	.0003	g
Citric acid	.0003	g
Vitamin B_{12}	.072	μg
Nitrate-free, millipore-filtered seawater	100	ml
pH after autoclaving	7.8–8.0	

The nitrogen-deficient culture was exposed to 1800 foot-candles of fluorescent illumination in a two-liter cotton-stoppered glass flask at 18 ± 2°C. After four days the cells had become visibly chlorotic. They were then placed in the dark for 15 hours, after which estimations were made of the cell count, cellular nitrogen, and chlorophyll. Enough KNO_3 solution was then added to give an initial concentration of 2.68 mg NO_3-N per liter, and the culture was placed in the light. Aliquot samples were removed at intervals for the estimation of nitrate, cellular nitrogen, and chlorophyll, carotenoid, and cell numbers.

The nitrogen-starved culture removed the added nitrate rapidly during the first six hours of illumination, and continued to assimilate nitrate at a slower rate during the remainder of the experiment (Table 1). The nitrate removed was never completely recovered as cellular nitrogen, suggesting either the formation of nitrogen compounds not recovered by the Kjeldahl digestions, or an excretion of extracellular nitrogen compounds into the medium. After 47 hours 93% of the added nitrate was removed, and 79% of this amount was recovered as cellular nitrogen. The nitrogen per cell increased rapidly at first (Fig. 1A), then became constant and declined thereafter. The values for nitrogen per cell after 26 hours agree with those found by Ketchum and Redfield (1949) for normal *Nitzschia closterium*[2] cells.

TABLE 1. *Changes in cell number, nitrogen, and pigments in an enriched culture of* Phaeodactylum tricornutum

Time hrs	Cells × 10^4/L	Residual NO_3-N mg/L	Cellular nitrogen mg/L	Chlorophyll *a* mg/L	Carotenoid SPU/L	Cellular nitrogen / Chlorophyll *a*	Carotenoid / Chlorophyll *a*
0	70	2.68	.21	.080	.050	2.64	.62
5	72	2.04	—	.090	.042	—	.47
6.5	73	1.69	.83	.091	.020	9.10	.22
23.5	100	1.02	1.34	.174	.060	7.72	.34
26.5	118	0.77	1.43	.201	.080	7.12	.47
29.5	135	0.48	1.48	.210	.100	7.05	.47
47.5	340	0.18	1.98	.302	.138	6.57	.46

Chlorophyll *a* per cell reached a maximum between 20 and 24 hours after enrichment and then declined (Fig. 2B). Carotenoid initially was high in the cells, then declined rapidly as chlorophyll *a* increased; however, carotenoid synthesis resumed after eight hours, reached a maximum at 28 hours, then declined. Hence the carotenoid:chlorophyll ratio was high at the start of the experiment, reached a low eight hours after enrichment, then increased (Fig. 2C).

The largest increase in chlorophyll *a*, carotenoid, and cell numbers occurred between six and 23 hours, which corresponded to the maximum increase in cellular nitrogen. The increase in the nitrogen per cell also corresponded with decreases in the carotenoid:chlorophyll *a* ratio. Highest values for chlorophyll *a* occurred when the nitrogen per cell was highest, while the pigment ratio (carot.:chl[a]) reached a minimum somewhat earlier than the other maxima. The ratio between cellular nitrogen and

[2] Probably *Phaeodactylum tricornutum*.

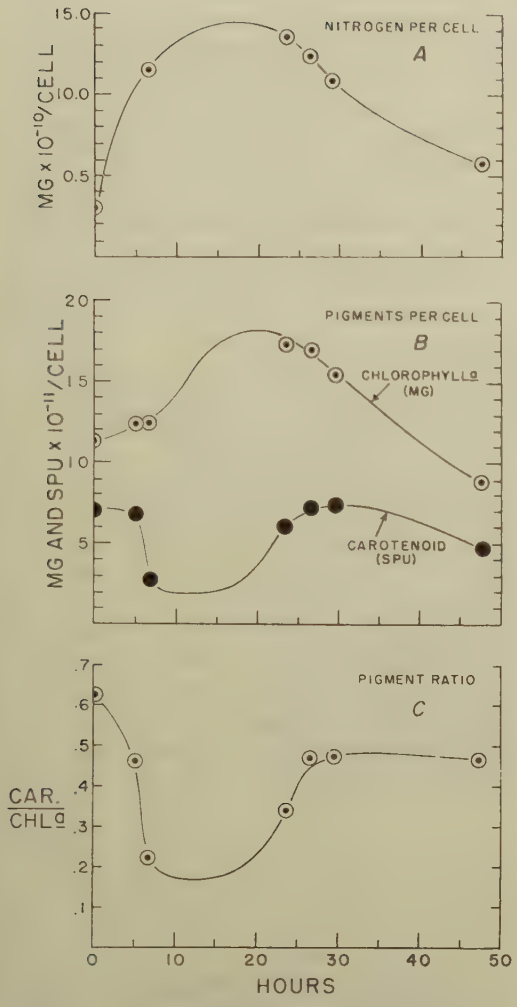

FIG. 1. Changes in chlorophyll *a*, carotenoid, and cell nitrogen in a culture of *Phaeodactylum tricornutum*.

chlorophyll *a* (Table 1) increased from 2.6 to 9.1 at 6.5 hours, and then decreased to values ranging from 7.72 to 6.57 for the remainder of the experiment.

Analyses of cellular nitrogen, chlorophyll *a*, and carotenoid have also been made on cultures of other species, and these are compared in Table 2 with some analyses made on natural populations. All N:Chl^a ratios for algal cultures fall in the range of 1.7–9.1. Young and senescent cultures of the green flagellate *Dunaliella* had ratios of 7.0 and 1.7, respectively. Nitrogen-to-chlorophyll values for the three diatom cultures, *Ditylum*, *Cyclotella*, and *Melosira*

TABLE 2. *Pigment nitrogen relationship in natural populations and cultures of marine phytoplankton*

Organisms	N:Chl^a	Carot.:Chl^a
Phaeodactylum tricoruntum		
Nitrogen deficient	2.64	.66
Nitrogen enriched	9.10	.22
Dunaliella euchlora		
Nitrogen deficient	1.70	.59
Nitrogen enriched	7.00	.40
Ditylum Brightwellii		
Stock culture	8.3	.29
Melosira sp.		
Stock culture	4.6	.67
Cyclotella sp.		
Stock culture	6.0	.59
Cosinodiscus sp.[1]		
Net haul	13.2	—
Goniaulax sp.[1]		
Net haul	14.7	—
Av. for natural population in Long Island Sound. Based on dry weight in net hauls.[1]	9.4	—
Natural populations of marine phytoplankton.[2]	6.3	—
Natural populations of marine phytoplankton.[3]	3.0	—

[1] Harris and Riley (1956)
[2] Krey (1953)
[3] Fleming (1940)

average 6.7. Harris and Riley (1956) determined particulate nitrogen and chlorophyll content of net hauls of *Coscinodiscus* and *Goniaulax* which give unusually high values for the N:Chl^a ratio. This is probably due to the unavoidable inclusion of detrital and zooplankton contaminates. Their average N:Chl^a ratio for all net hauls in Long Island Sound was 9.4 on a dry weight basis. Nitrogen-to-chlorophyll ratios given by different workers for "marine phytoplankton" vary considerably. Computed from protein, Krey's (1953) average value is 6.3, while Fleming's (1940) ratio is 3.0.

In culture experiments the cells become visibly chlorotic as the deficiency of nitrogen develops. This is reflected by an increase in the ratio of carotenoid:chlorophyll *a*, and by a closely correlated decrease in the N:Chl^a ratio. These ratios are compared in Figure 2, which includes not only the experimental results with *Phaeodacylum tricornutum* but also data from cultures of

other species. Except for two aberrant values these results are related by the equation[3]:

$$N/Chl^a = 11.4 - Carot./Chl^a \quad (1)$$

The two aberrant points in Figure 2 were for grossly nitrogen-deficient cultures. It seems probable that the extreme nitrogen deficiency induced in these cultures is never duplicated in nature. Numerous Carot.: Chl^a ratios observed by the senior author for natural populations of marine phytoplankton in Atlantic coastal waters have ranged from 0.15–0.55. The most deficient cultures had pigment ratios of 0.59–0.67, suggesting that nitrogen-starved cultures become more chlorotic than natural populations in the sea.

NITROGEN CONTENT OF NATURAL POPULATIONS

We have made measurements of particulate nitrogen, chlorophyll, and carotenoid in Vineyard Sound and the Atlantic coastal slope waters. The observed total particulate N:Chl^a ratios in Vineyard Sound are approximately five times larger than any of the values obtained in our culture experiments (Table 3), suggesting a large amount of non-phytoplankton nitrogen. The ratios in the coastal and slope waters are closer to our culture values.

The ratio of N:Chl^a in the phytoplankton was calculated from the observed pigment ratio by Equation (1). The former ratio was then multiplied by the chlorophyll content in order to estimate the portion of the total particulate nitrogen which was contained in the phytoplankton. In the Vineyard Sound populations the carotenoid: chlorophyll ratios ranged between 0.23 and 0.18, giving calculated N:Chl^a ratios of 8.6 to 9.1 for the phytoplankton fraction of the particulate nitrogen. The carotenoid: chlorophyll values for the coastal and slope waters were 0.37–0.27, giving calculated N:Chl^a ratios of 7.3 to 8.3. The calculated phytoplankton nitrogen comprised 13 to 25% of the total in Vineyard Sound, 43 to 58% at the inshore stations on the conti-

[3] Nitrogen and chlorophyll *a* in milligrams, carotenoid in SPU units.

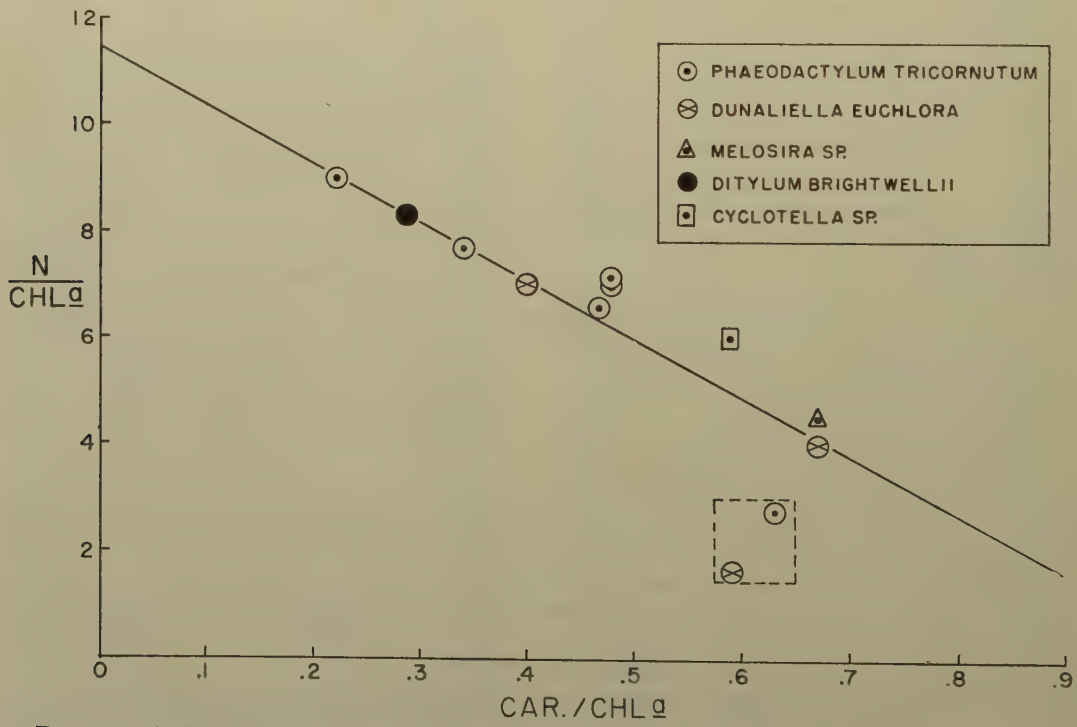

FIG. 2. Relationship between Nitrogen:Chl^a and Carotenoid: Chl^a in 5 cultures of marine phytoplankton.

TABLE 3. *Percentage of organic nitrogen in phytoplankton in surface waters*

Location	Date	Particulate nitrogen μg/L	Chl[a] mg/m³	Carot./Chl[a]	Observed N:Chl[a]	Calculated* N:Chl[a]	Phytoplankton nitrogen μg/L	Phytoplankton nitrogen % total
Vineyard Sd.	7/2/57	140.0	2.73	0.18	51.3	9.1	25.0	18.0
Vineyard Sd.	7/10/57	74.0	1.54	0.23	48.2	8.6	13.4	18.1
Vineyard Sd.	7/23/57	94.0	2.00	0.20	47.0	9.0	18.0	19.2
Vineyard Sd.	7/31/57	57.0	1.50	0.22	38.0	8.7	13.1	23.1
Continental Shelf Sta. U, 25 fath.	11/14/57	53.1	3.75	0.27	14.2	8.3	31.1	58.5
Sta. N, 100 fath.	11/14/57	36.0	1.86	0.27	19.3	8.3	15.5	43.0
Sta. G., 1000 fath.	11/14/57	4.1	0.39	0.37	10.4	7.3	2.8	70.0

* From relation given by Equation (1).

nental shelf, and 70% for offshore stations. This general increase with increasing distances from land reflects a diminution of non-phytoplankton bacterial and detrital nitrogen.

DISCUSSION

Our experiments show that nitrogen deficiency produces a simultaneous decrease in both cellular nitrogen and chlorophyll. Thus the hydrolysis of cytoplasmic and chloroplastic proteins appear to take place concurrently, or nearly so, and the loss in chlorophyll may be attributed to the decomposition of the pigment protein complex. Therefore, the synthesis or decomposition of chlorophyll is closely associated with nitrogen metabolism, and any environmental factor which modifies nitrogen metabolism may be expected to have an associated influence on the chlorophyll content.

The onset of nitrogen deficiency is accompanied by a gradual loss of carotenoid as well as chlorophyll. Since the loss of the chlorophyll is much faster the ratio of the pigments changes. Our field observations show that in general inshore natural populations have lower pigment ratios than do offshore populations. Furthermore, in the surface waters, diurnal changes in the pigment ratios have been observed which could be interpreted to mean, from the results reported in the paper, that cellular nitrogen also varies with changing light intensity. Harvey (1953) has observed that the nitrogen per cell in cultures decreased as the light intensity was increased. He suggested that phytoplankton might be nitrogen deficient during the day and make up the deficiency at night.

The percentages of the total particulate nitrogen attributed to the phytoplankton in this paper are considerably higher than values given by Fox *et al.* (1952). This discrepancy is undoubtedly due to differences in techniques used to concentrate particulate matter. It is our opinion that Fox *et al.* (1953) have not definitely shown that their method of filtration is not adsorbing dissolved materials. We have also measured total amine-amide nitrogen which includes the dissolved organic matter. If our values for phytoplankton nitrogen were compared with the total amine-amide nitrogen rather than with particulate nitrogen the percentage of nitrogen in the plants would be lower and in agreement with the data of Fox *et al.* (1952). If particulate nitrogen is defined as the material retained by von Brand's (1935) flocculation method then the phytoplankton fraction is always sizable.

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The Production of Extracellular Carbohydrates by Some Marine Flagellates[1]

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ABSTRACT

Extracellular carbohydrate production during the growth of bacteria-free cultures of the marine flagellates *Isochrysis galbana*, *Monochrysis lutheri*, *Prymnesium parvum*, *Dunaliella euchlora*, *Pyraminomonas* sp., *Rhodomonas* (?) sp., and *Chlamydomonas* sp. was followed by the N-ethyl carbazole method.

Carbohydrate production did not parallel cell numbers during exponential growth of any of these organisms; maximum concentrations during this phase were under 3 mg/L. Carbohydrates accumulated in all stationary cultures (having 10^6–10^7 cells/ml), to the greatest extent in *Prymnesium* cultures, which attained 123 mg/L. *Isochrysis* and *Monochrysis* produced 25 and 16 mg/L, respectively, while the others had under 10 mg/L even when cell disintegration was evident.

Aging cultures (not all bacteria-free) of 4 diatoms, 2 dinoflagellates, 2 additional green algae, and a cyanophyte were assayed. Concentrations ranged from 1.5 to 60 mg/L.

This suggests that the organisms studied contribute significant free carbohydrates only during late bloom conditions, when algae are abundant and under physiological circumstances resembling stationary or declining culture.

INTRODUCTION

Increasing investigation of the occurrence and nature of dissolved organic substances in natural waters makes parallel study of the formation of such materials in pure cultures of microorganisms increasingly pertinent. We are concerned in particular with reports of substances in sintered glass or Millipore filtered sea water which react as do carbohydrates with sulfuric acid and either anthrone or N-ethyl carbazole. Collier *et al.* (1953), Collier (1958), and Wangersky (1952) found such substances in waters of the Gulf of Mexico. The maximum concentrations recorded were of the order of 100 mg/L, but more usual values lay between 1 and 25 mg/L. Pacific Coast water, according to Lewis and Rakestraw (1955), contained less than 0.7 mg/L, but the concentration in coastal lagoons was as high as 7.9 mg/L.

A number of studies of the production of extra-cellular carbohydrates in pure cultures are discussed by Lewin (1956). The organisms so far investigated in detail either have polysaccharide sheaths or capsules, or else cell walls, which may be cast off at cell division and thus contribute to the extracellular material, soluble or particulate. The present report concerns the existence of soluble carbohydrates in pure cultures of seven marine or brackish water photosynthetic flagellates. All except a *Chlamydomonas* sp. are naked. A few samples of other organisms were analyzed also, partly to test the method.

The primary question is whether or not measurable carbohydrates are produced, and if so, to what extent during different phases of growth of the culture. By "soluble car-

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bohydrate" is meant material that passes through an HA Millipore filter and yields a red-violet color with N-ethyl carbazole.

METHODS

Algal cultures

Except for stock cultures, algae were grown in 300- to 500-ml lots in one-liter flasks, aerated with sterile 1% CO_2 in air, and maintained at 20–21°C under constant illumination of ca. 500 foot-candles. The medium was Long Island Sound sea water, salinity 25–27‰, enriched as follows, and autoclaved:

NH_4Cl	50 mg/L unless specified
NaH_2PO_4	20 mg/L
Ferric sequestrene (NaFe EDTA, 13% iron)	10 mg/L
Cu	0.005 mg/L
Zn	0.01
Co	0.005
Mn	0.1
Mo	0.005 mg/L
Thiamine	0.2 mg/L
Biotin	1 μg/L
B-12	1 μg/L

Ammonia rather than nitrate was used because the latter interferes with the N-ethyl carbazole reaction. (In old cultures the level may become so low that no interference occurs.) *Isochrysis* and *Rhodomonas* were sensitive to ammonia, and hence no more than 50 mg/L was used; 150 mg/L was used for the *Chlamydomonas* sp., and for *Monochrysis* in one experiment.

Sterility tests were made with the above medium enriched further with 1 g/L of both peptone and glucose, also using a slight modification of medium STP of Provasoli *et al.* (1957). Algal cultures were also examined directly with phase contrast. Cells were killed with I_2-KI-acetic acid and counted in a hemocytometer.

The derivations of cultures of *Isochrysis galbana*, *Monochrysis lutheri*, *Prymnesium parvum*, *Dunaliella euchlora*, *Chlorococcum* sp., and *Chlorella* # 580 are given in Davis and Guillard (1958). Pure cultures of *Amphidinium carteri*, *Rhodomonas* (?) sp., and *Pyraminomonas* sp. were kindly furnished by Dr. Luigi Provasoli and Dr. John McLaughlin of the Haskins Laboratories, New York. (The latter algae were originally isolated, respectively, by one of the authors (R. G.) from Falmouth Great Pond, Mass.; by R. Lasker from the Gulf Stream; and by R. Lewin from a rock pool near San Francisco.) *Chlamydomonas* "Y", *Cyclotella* sp., *Nitzschia brevirostris* Hustedt, and *Melosira* sp. were isolated in pure culture from West Tisbury Great Pond, Massachusetts, by one of us (R. G.).

Preparation of samples

Because our experience indicates that sample preparation—that is, removing cells from the medium—limits the over-all accuracy of the method, a detailed account of the procedure is given.

Aliquots of the algal cultures were filtered through autoclaved HA Millipore filters and maintained aseptically in a freezer overnight for analysis. Clogging of the filter by dense cultures limited sample size to less than 10 ml. In such small aliquots, adsorption or contamination by the filters might be significant. Using glucose solutions of 1–5 mg/L, we were unable to demonstrate significant adsorption in 5-ml aliquots drawn through filters. However, it was found necessary to wash the Millipore filter with ca. 1 liter of distilled water to remove small but significant quantities of material giving a positive reaction for carbohydrate. (This contamination might be insignificant in large samples.) A sea water blank was drawn through each filter before the sample. There was good agreement between duplicate aliquots drawn through separate filters. However, replicate aliquots of algal cultures drawn through the same filter agreed well only when the cultures were not extremely dense. Using cultures so dense that less than 10 ml would clog the filters, it was found that the first 3–4 ml aliquot had significantly less measurable carbohydrates than the following ones. The increase is attributed to leaching from cells injured by the vacuum filtration. This is supported by observations on cells injured in other ways. Sea water drawn through a filter after an aliquot of algae did not always contain measurable carbohydrates, but distilled water so filtered invariably did. Killing

cells by heating to 55°–60°C generally released large amounts of carbohydrate. However, some organisms, notably *Monochrysis lutheri*, produce a carbohydrate-like material which is either volatilized or destroyed by heat.

Inoculation also causes the release of small amounts of carbohydrate. Freshly inoculated medium, previously carbohydrate-free, was found to have 10–100 times more carbohydrate than could be accounted for by the amount in the inoculum. The concentration was lower in 24 hours. In an experiment with *Dunaliella* (in triplicate) the quantity fell from 0.7 mg/L to an undetectable level in one hour. This has not been investigated further. Proctor (1957) discusses similar phenomena associated with the lysis of algal cells upon inoculation and during the course of culture.

In a few trials, cells were removed from their medium by centrifugation (RCF of ca. 1000) and the resulting medium filtered and compared with samples clarified in small aliquots by filtration only. There were no significant differences. We adopted as the best practical procedure filtering samples small enough to pass through the filter without observable slowing of the filtration rate. Thus, only 2–4 ml of the densest samples were filtered. General reliability of the method is indicated by the fact that such small aliquots of fairly dense cultures often contained almost no measurable carbohydrate. These results are consistent with the findings of Lasker and Holmes (1957), who investigated the loss during filtration of carbon-14 fixed by algal photosynthesis.

The samples were not tested for the presence of bacteria. The techniques used are those routinely employed for handling serum and organic media which are not autoclaved. No difficulty with contamination was observed. In aliquots of samples deliberately contaminated with bacteria, the measured carbohydrates increased, rather than decreased, suggesting that bacteria synthesize carbohydrates from other organic materials produced by the algae. Observations by Tolbert and Zill (1956) and Allen (1956) suggest that organic acids such as glycollic may in part be responsible. Glycollate does not give a positive N-ethyl carbazole reaction.

Analysis for carbohydrates

The method is essentially that used by Collier *et al.* (1953) and Lewis and Rakestraw (1955), with two minor modifications. Glucose was used as the reference standard instead of arabinose, and the sample was covered with a thin film of mineral oil immediately after addition of the reagent, to prevent oxidation. Mineral oil was substituted for the xylene or paraffin originally recommended because both of these substances occasionally contain impurities which interfere with the carbohydrate reaction.

RESULTS

Simultaneous measurements of cell numbers and carbohydrate concentrations were made at intervals during the growth of cultures of the seven flagellates. Figure 1 illustrates these data for a single culture of *Monochrysis lutheri*. In Figure 2 are plotted the averages of two measurements made from each of two cultures of *Isochrysis galbana*, grown concurrently. Replicates of

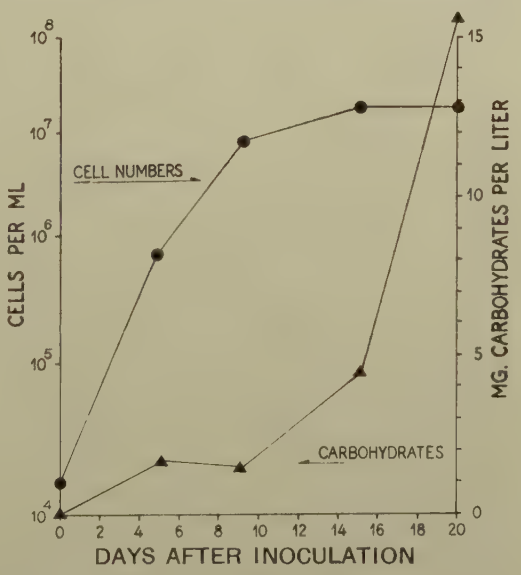

FIG. 1. Cell density and carbohydrate concentration during growth of a culture of *Monochrysis lutheri*.

Monochrysis and *Dunaliella euchlora* were run consecutively, hence the data are presented separately. Table 1 gives the results of seven experiments besides those shown in the figures.

The simplest course of carbohydrate production is shown in Figure 1. During the logarithmic phase of growth, while cell numbers increased approximately a thousandfold, the carbohydrate level remained relatively constant and less than 2 mg/L. As the culture entered the stationary phase, carbohydrates began to accumulate. Between the ninth and twentieth days, while cell numbers barely doubled, the carbohydrates increased about ten-fold Figure 2 is generally similar, but there is an exceptionally high value for carbohydrates on day 13, the first day of the stationary phase of growth. This occurred in both cultures. Table 1 shows similar irregular highs in one of each of the replicate experiments with *Monochrysis* and *Dunaliella*, also in the experiments with *Pyraminomonas* sp. and *Prymnesium parvum*.

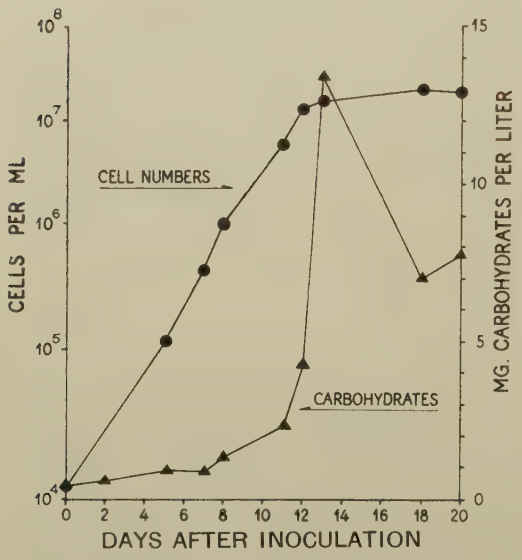

FIG. 2. Cell density and carbohydrate concentration during growth of two cultures of *Isochrysis galbana*. Average values are plotted. Each culture was sampled in duplicate for carbohydrates.

It is not known whether these irregular high values result from accumulation of carbohydrate followed by assimilation, or whether they are in effect artifacts, due perhaps to increased sensitivity of the cells to filtration at that particular culture phase.

In no instance did the carbohydrate level rise above 3 mg/L in an actively growing culture. Accumulation occurred only in stationary or declining cultures.

TABLE 1. *Cell numbers and carbohydrate concentrations during the growth of seven cultures*

Day	*Dunaliella euchlora*		*Dunaliella euchlora*		*Pyraminomonas* sp.		*Chlamydomonas* "Y"		*Monochrysis lutheri*		*Prymnesium parvum*		*Rhodomonas* (?) sp.	
	Cells 10^4/ml	Carbohydr. mg/L	Cells 10^4/ml	Carbohydr. mg/L	Cells 10^4/ml	Carbohydr. mg/L	Cells 10^4/ml	Carbohydr. mg/L	Cells 10^4/ml	Carbohydr. mg/L	Cells 10^4/ml	Carbohydr. mg/L	Cells 10^4/ml	Carbohydr. mg/L
0	1.63	0	1.36	0	0.18	0	2.36	0	2.15	0	1.75	0	1.4	0
1										1.7				
2			5.7	0.5	1	0.9	6.5	0.5			9.7	0.2	9.7	1.1
3									32	0.8	27	0.8	14	0.2
4	22.6	0	85	0.7	2.5	0.6			109	1.1	105	1.7	30	1.4
5							66	2.0						
6	55.0	1.5	153	1.0	22	0			750	1.7	500	2.8	32	1.4
7	125	0.5	193	1.8	44	1.1			1580	1.7	650	5.8	82	0.6
8			272	3.1	105	2.8							78	1.6
9			330	2.9	122	3.4	625	2.1	1900	7.0	775	15.9	154	1.9
10			310	5.9	100*	1.6			1900	2.6	650	123	†	6.4
11	150	0.8												
13	160	1.4	310	4.0	77*	4.2			2100	7.4	415	31.7	†	8.8
15							590	2.1						
18	175*	1.8												
20							620	10.6						
26	200*	5.1												

* Disintegrating cells observed.

† Accurate count of living cells impossible.

TABLE 2. *Maximum carbohydrate concentrations observed in cultures of various organisms*

Organism	Highest carbohydrate concentration found (mg/L)
Prymnesium parvum	123
Isochrysis galbana	25
Monochrysis lutheri	15.7
Actinocyclus sp.*	18.1
Cyclotella sp.	1.5
Nitzschia brevirostris†	25.6
Melosira sp.	60
Dunaliella euchlora	9
Pyraminomonas sp.	5.4
Chlorella sp. (Ind. U. #580)	9
Chlorococcum sp.	27
Chlamydomonas sp.	10.6
Gymnodinium sp.‡	4.4
Amphidinium carteri	§
Synechococcus sp.‡	35
Rhodomonas (?) sp.	8.8

* Bacteria present; mucilaginous even in pure culture.

† Tentative identification.

‡ Bacteria present.

§ Undetermined due to interference, but over 5.

The maximum levels attained varied considerably with the species. Table 2 shows maximum values found either during the course of the experiments described or in samples taken from old stock cultures. Members of the Chyrsophyta were the highest producers; the greatest value attained, 123 mg/L, was in a culture of *Prymnesium*. Note, however, that one centric diatom, *Cyclotella* sp. (*meneghiniana* ?), accumulated only 1.5 mg/L in a declining culture. No measurable carbohydrates were produced up to that point in this particular culture. The highest concentration attained by a green alga was 27 mg/L, in a stock culture of *Chlorococcum* sp. Both dinoflagellates produced measurable quantities of carbohydrates, but it was necessary to dialyze the samples to remove interfering substances. (Collier 1958 found about 5 mg/L in an impure culture of a *Prorocentrum* sp.) The cryptomonad studied, *Rhodomonas* (?) sp., accumulated only 8.8 mg/L in a culture in which considerable cell disintegration was evident. Old cultures of the blue-green alga *Synechococcus* sp. became mucilaginous; one such, not bacteria-free, had 35 mg/L.

DISCUSSION

The course of carbohydrate production in cell-free medium did not parallel cell numbers in cultures of any of the organisms studied. Such accumulation as occurred began with the end of the logarithmic phase of growth and, in most instances, increased during the stationary phase or senescence of the cultures. The fact that killing cells liberates comparatively large amounts of carbohydrate supports the idea that its presence, particularly during the phase of active growth, is due largely to the death of cells. While from a physiological point of view it would be desirable to know if healthy cells do in fact "leak" a small amount of carbohydrate, from the ecological view it makes little difference, because some cells die even under the most favorable circumstances. Note that in the case of *Prymnesium*, the release of leucosin droplets as described by Carter (1937) offers another possible mechanism for carbohydrate accumulation.

The fresh water diatom *Navicula pelliculosa* studied by J. C. Lewin (1955) showed a similar pattern of extracellular carbohydrate production, accumulating capsular polysaccharide under conditions of mineral deficiency when photosynthesis was allowed to continue. We also have observed that pure cultures of certain centric and pennate diatoms become mucilaginous as the cultures age.

On the other hand, it has been shown that this pattern is not universal. Allen (1956) and R. A. Lewin (1956) found that various green algae, mostly *Chlamydomonas* species, produced extracellular polysaccharides and other compounds throughout the course of growth. In addition, Fogg (1952) found a similar pattern of liberation of nitrogenous compounds by *Anabaena cylindrica*.

Nevertheless, the evidence available, *e.g.*, Collyer and Fogg (1955), indicates that it is during the stationary phase of culture that algae tend to accumulate photosynthetic reserves. It is unlikely that external carbohydrates would be used as an energy source under such circumstances; hence if only moderate amounts are found in the medium at this time, it implies that reserve products

are either conserved or consist largely of other materials, such as polypeptides or organic acids.

The ecological implication of the present report is that the flagellates studied can be expected to contribute materially to the filterable carbohydrates of natural waters only under late bloom or post-bloom conditions. Some of these organisms may occur in great numbers at times. Droop (1954) reports finding concentrations of *Monochrysis lutheri* and *Hemiselmis* sp. of the order of 10^7 cells/ml in rock pools. *Prymnesium parvum* blooms of about 5×10^5 cells/ml are mentioned, *e.g.*, by Reich and Aschner (1947). The experimental data indicate that such phytoplankton densities might yield carbohydrate concentrations of the order of 10 mg/L.

However, such concentrations of organisms, particularly of single species, are of relatively limited occurrence. Hulbert (1956) and Smayda (1957) found total phytoplankton maxima of the order of 10^5 cells/ml in estuarine waters; citations given by the latter author indicate that maxima in open neritic waters are an order of magnitude lower. Assuming that these organisms behave like stationary cultures, the maximum amount of carbohydrate liberated would not exceed one mg/L. The possibility exists that the organisms might die under some circumstances more rapidly than they do in culture, thus liberating more. Note also that the relative sizes of different organisms, thus the total quantity of phytoplankton, have not been considered.

Data relating carbohydrate concentrations in nature to phytoplankton composition and physiological condition would be extremely desirable. The evidence that compounds other than carbohydrates form a significant fraction not only of algal exudates but of the total organic carbon in natural waters suggests strongly that these materials be investigated concurrently with carbohydrates.

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Bioassay of Water from a Concretion-Forming Marl Lake

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ABSTRACT

Using a pure, bacteria-free culture of *Nostoc muscorum* as a bioassay test organism, it was found that the water of Bass Lake, Livingston Co., Michigan, had a severe phosphate deficiency and less severe deficiencies of K, Fe, Mn, and Mg. Emission spectrographic analyses showed that the concretions had Ca as the principal constituent, Mg as the major impurity, and Fe, Si, Mn, Al, and Sr as minor impurities. When boiled, the lake water formed a white precipitate of $CaCO_3$, which could be prevented by the addition of a very small amount of K_2HPO_4. It is suggested that concretion formation in marl lakes is associated with extreme phosphate deficiency.

INTRODUCTION

The factors accounting for the low productivity of a concretion-forming marl lake were explored by Raymond (1937). He studied the factors of lake depth, total electrolytes, water color and turbidity, dissolved oxygen, pH, and the CO_2 content. He felt that the water might have been deficient in certain elements required for plant and animal growth, but he made no chemical analyses of the water.

In the present investigation, water from Bass Lake was bioassayed in order to determine whether the lake water was deficient in any nutrient elements. Bass Lake is one mile southwest of Lakeland, Livingston Co., Michigan, and is the concretion-forming marl lake on which Raymond (1937) based his study. *Nostoc muscorum* was chosen as the organism for the bioassay, chiefly because of the author's (Eyster 1951, 1952, 1953, 1958) familiarity with both its culture and its nutritional requirements. The fact that the water was alkaline in pH and supported a blue-green alga around which concretions formed also contributed to the decision for using the blue-green alga, *Nostoc muscorum*, as the bioassay organism.

The emission spectrographic analyses of the lake concretions were kindly done by Howard Bales of the Charles F. Kettering Foundation. Dr. Walter F. Morofsky, Director, Kellogg Gull Lake Biological Station, is acknowledged for his courtesy in granting the author and his family living accommodations at the Biological Laboratory. This made it convenient for the author to examine Fish Lake, another concretion-forming marl lake located near Orangeville, Barry Co., Michigan.

EXPERIMENTAL

Samples of water from Bass Lake were collected in polyethylene bottles on October 2, 1955. Two types of bioassays were performed. In the first type, known macronutrients and micronutrients were added separately to successive aliquots of lake water in 250-ml Erlenmeyer flasks. The concentration of each nutrient was similar to that in the medium used by Chu (1942), which as here modified had 27.2 mg $CaCl_2$, 39 mg NH_4NO_3, 10 mg K_2HPO_4, 25 mg $MgSO_4 \cdot 7H_2O$, 100 mg $NaHCO_3$, 1.37 mg $FeSO_4 \cdot 7H_2O$, and 1 ml A_5(B, Cu, Mn, Mo, Zn) trace element supplement per liter. Each constituent of the nutrient solution was prepared as a separate stock solution 100 times the strength desired, using "Specpure" reagents (Johnson Matthey Ltd., Hatton Garden, London, England). In successive cultures one milliliter of each stock nutrient was diluted to 100 ml with lake water. The flasks containing the lake water and nutrient supplements were cotton-plugged, autoclaved, and inoculated aseptically with one loopful of a pure actively growing liquid culture of *Nostoc muscorum*. The cultures were grown statically with constant illumination of approximately 100 ft-c fluorescent

light at about 25°C. After 57 days the total growth was measured by determining the packed cell volume. Fifty-milliliter aliquots were centrifuged, and the concentrated cells were transferred to Exax hematocrit tubes (Cenco). Cultures with dense growth, *i.e.*, the complete and the K_2HPO_4 supplements, required the use of only 10-ml aliquots. Centrifuging the tubes for 30 minutes at 3300 rpm at a radius of 22 cm produced a constant packed cell volume.

In the second type of bioassay, the complete supplement was added to the lake water, and a different nutrient was omitted in successive cultures. The culturing was generally similar to the first type of bioassay. Packed cell volumes were determined after 49 days of growth. Ten-ml aliquots were used for dense cultures and 50-ml aliquots for sparse cultures.

RESULTS

The results of the first type of bioassay are given in Table 1. It is evident that the lake water was severely deficient in factors needed for the growth of *Nostoc muscorum*, and that the growth of the alga was markedly increased only by the addition of K_2HPO_4, and even more by the addition of all the nutrients known to be required by the alga.

Table 2 gives the results of the second type of bioassay, which was an improvement over the first because mild or intermediate deficiencies as well as severe deficiencies were revealed by it. One could also evaluate the relative amount of deficiency from the results of the second bioassay by assuming 100% yield in the lake water having the complete nutrient supplement. Approximate evaluations of the less severe deficiencies were: Fe, 42%; A_5, 22%; and Mg, 13%.

Further experimentation (Table 3) showed that potassium as well as phosphate was deficient in the lake water. The potassium deficiency reduced the yield of the lake water by 43%, whereas there was almost no growth at all in the absence of phosphate. Hence, it might be said that phosphate is the primary deficiency but that potassium is a significant secondary deficiency. Of the A_5 constituents (Table 4), only manganese was deficient in the lake water.

Emission spectrographic analyses have shown that the concretions had Ca as the principal constituent, Mg as the major impurity, and Fe, Si, Mn, Al, and Sr as minor impurities.

The lake water had a pH of 7.8, a phenolphthalein hardness equivalent to 6.8 ppm

TABLE 1. *First type of bioassay analysis of Bass Lake water based on 57-day-old cultures of* Nostoc muscorum

Supplement added to lake water	Yield μL/m
None	0.11
NH_4NO_3	0.13
$MgSO_4$	0.13
$CaCl_2$	0.10
$FeSO_4$	0.11
$MnSO_4$	0.12
A_5	0.07
$NaHCO_3$	0.10
K_2HPO_4	1.50
All (complete)	3.60

TABLE 2. *Second type of bioassay analysis of Bass Lake water based on 49-day-old cultures of* Nostoc muscorum

Supplement added to lake water	Yield μL/ml	Growth reductions
None	0.08	
Complete	3.60	0%
Complete less K_2HPO_4	0.10	98%
Complete less $MgSO_4$	3.40	
Complete less Mg [$(NH_4)_2SO_4$ added]	3.15	13%
Complete less NH_4NO_3	3.45	
Complete less $CaCl_2$	3.65	
Complete less $FeSO_4$	2.10	42%
Complete less A_5	2.70	22%
Complete less Na	3.65	

TABLE 3. *Further bioassay analysis of Bass Lake water designed to characterize the primary deficiency and based on 13-day-old cultures of* Nostoc muscorum

Supplement added to lake water	Yield μL/ml	Growth reductions
None	0.04	99%
Complete	2.90	0%
Complete less K_2HPO_4	0.14	95%
Complete less K (Na_2HPO_4 added)	1.65	43%
Complete less PO_4 (KCl added)	0.07	98%

TABLE 4. *Further bioassay analysis of Bass Lake water designed to characterize the A_5 deficiency and based on 51-day-old cultures of* Nostoc muscorum

Supplement added to lake water	Yield µL/ml
Complete	5.40
Complete less A_5	1.30
Complete less A_5 + Mn	4.50
Complete less A_5 + Mo	1.15
Complete less A_5 + B	0.95
Complete less A_5 + Zn	0.95
Complete less A_5 + Cu	1.20

$CaCO_3$, and an additional methyl orange hardness equivalent to 145 ppm $CaCO_3$. When boiled the water formed a white precipitate (of $CaCO_3$) according to the following equation:

$$Ca(HCO_3)_2 \xrightarrow{\text{boiled}} CaCO_3 + CO_2\uparrow + H_2O$$

The addition of K_2HPO_4 to Bass Lake water prevented the formation of a white precipitate (of $CaCO_3$) according to the following equation:

$$\underset{\text{(dissolved)}}{CaCO_3} + K_2HPO_4 \rightarrow K_2CO_3 + CaHPO_4$$

There was a shift in the pH from 7.85 to 8.35, indicating that K_2CO_3 rather than $KHCO_3$ had been formed.

A fairly saturated solution of $Ca(HCO_3)_2$ was prepared and then titrated to determine its strength. Measurements were made of the pH of the boiled solution of $Ca(HCO_3)_2$ and of boiled aliquots of a $Ca(HCO_3)_2$ solution to which progressively larger amounts of K_2HPO_4 had been added. The data are given in Table 5. The $Ca(HCO_3)_2$ solution had an approximate strength of 0.25 m-mole/100 ml. When boiled, the solution showed a white precipitate and had a pH of 7.85. The addition of merely 0.0006 m-mole of K_2HPO_4 to 100 ml of $Ca(HCO_3)_2$ solution prevented the formation of the precipitate of $CaCO_3$ upon boiling, and caused an abrupt shift in pH to 8.35. Phenolphthalein also verified this shift in pH. The former solution without any phosphate was colorless in the presence of phenolphthalein, whereas the addition of 0.0006 m-mole of K_2HPO_4 caused the boiled solution to give a distinctly red

TABLE 5. *The pH of boiled $Ca(HCO_3)_2$ solutions, 0.25 m-mole/100 ml, to which varying amounts of K_2HPO_4 had been added*

m-mole K_2HPO_4 added to 100 ml $Ca(HCO_3)_2$ solution	pH	Remarks
None	7.85	White ppt
0.0006	8.35	Clear soln
0.003	8.35	Clear soln
0.006	8.40	Slight ppt
0.012	8.50	More ppt
0.06	8.55	Much ppt

test with phenolphthalein. Precipitates were formed in the $Ca(HCO_3)_2$ solutions to which more than 0.006 m-mole K_2HPO_4 had been added. It seems that the precipitate in these solutions may have been $CaHPO_4$, which precipitated once the amount formed exceeded the solubility.

How can a small amount of phosphate prevent the precipitation of calcium carbonate? Titration values and pH determinations of fresh and one-day-old calcium bicarbonate solutions produced results which seemed to fit into a rational pattern. The phosphate-free calcium bicarbonate solution and the calcium bicarbonate solution with 0.0006 m-mole K_2HPO_4 were similar both in pH (7.45) and in titration value (0.5 mE/100 ml). When boiled and cooled in stoppered test tubes where there was a minimum of chance to absorb carbon dioxide from the atmosphere, the respective pH values were 7.85 and 8.35, and the titration values were approximately equal at 0.238 mE/100 ml. On standing one day in an open flask with ample surface exposure the pH values were the same (7.8) but the respective titration values were 0.289 mE/100 ml and 0.337 mE/100 ml. Hence, the phosphate had caused a greater reabsorption of carbon dioxide from the atmosphere thereby tending to keep calcium in solution as calcium bicarbonate.

DISCUSSION

Theories of lime precipitation in fresh water are discussed by Rankama and Sahama (1950), who explained that the precipitation of lime involved the removal of carbon dioxide by its physical escape from the water into the atmosphere and its con-

sumption by aquatic plants. Factors of temperature, partial pressure of carbon dioxide in the water, pH, and the content of other dissolved substances all are known to play a part in the precipitation of calcium carbonate in natural waters. Davis (1900) discussed the possible relation of vegetation to marl formation, and Johnston and Williamson (1916) emphasized that the purely physical factors should also be taken into account in discussions of its mode of deposition. Tilden (1897) found that $CaCO_3$ precipitation took place only where plants occurred and not indiscriminately upon every object exposed to the action of water. Some other references which ascribe marl formation to algae (blue-green, *Chara*, etc.) are Penhallow (1896), Glock (1923), and Dachnowski-Stokes and Allison (1928). Seemingly, no one has ever suggested that marl formation by plants may be related to extreme phosphate deficiency. However, Hastings *et al.* (1927) have reported that phosphate in solutions brought about a condition of apparent supersaturation of dissolved $CaCO_3$.

This investigation strongly suggests that concretion formation in marl lakes is associated with extreme phosphate deficiency. The concretions contain blue-green algae. Their photosynthetic activity causes the removal of CO_2 from $Ca(HCO_3)_2$, thereby producing a molecule of $CaCO_3$ for each molecule of CO_2 absorbed. The removal of CO_2 from the $Ca(HCO_3)_2$ by boiling or by photosynthesis should make no difference in the results. A round concretion of $CaCO_3$ is formed for each active mass of blue-green algae. The formation of jackets of $CaCO_3$ around the green "stems" of *Chara*, also, might require extreme phosphate deficiency.

This paper establishes two facts which relate the effect of phosphate to marl formation by plants. First, nutritional amounts (0.0006 m-mole) of K_2HPO_4 resulted in an autoclaved lake water nutrient medium which consistently had less precipitate than an autoclaved nutrient medium lacking phosphate. Second, boiled medium containing nutrient amounts of phosphate had a higher pH, which caused a greater reabsorption of carbon dioxide from the atmosphere.

In order to understand the variations which exist among lakes, it is necessary to have a knowledge of the physical and chemical factors involved in the metabolism of these lakes. It has been difficult both to measure some of these factors and to decide just when they become limiting (Rawson 1939). Many of these difficulties are not encountered in the bioassay method of analysis.

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Lake Water and Sediment

IV. Radiophosphorus Equilibrium with Mud, Plants, and Bacteria under Oxidized and Reduced Conditions[1]

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ABSTRACT

The phosphorus equilibration pattern and rate between mud and water was the same in natural Jenkin sampler cores, in artificial cores, and in bottles in which dredged surface mud was packed by centrifuge. Thus any specific natural physico-chemical or bacteriological layering of the surface muds of lakes is relatively unimportant in phosphorus exchange. Phytoplankton or bacterial cells equilibrate within a few minutes after addition. When antibiotics are used the P^{32} remains as inorganic PO_4 and is rapidly taken up by higher plants, or in the absence of plants, there is a rapid loss of P^{32} to the mud. With one exception, in over 100 artificial systems tested, the amount of P^{32} remaining in the water at equilibrium was greater in the presence of bacteria than where antibiotic had been added. This was true whether the system was treated with nitrogen or air, *i.e.*, was aerobic or anaerobic. After a week less than 10% of the P^{32} remained in the water of an antibiotic treated sample, while two thirds remained in the control.

The remarkable ability of bacteria to hold phosphorus in the water might be accomplished in two ways:

1) By an acceleration of the rate of P^{32} return from the sediment to the water by bacteria in the mud. In all experiments while the turnover time of water was generally of the same order of magnitude for all systems, the turnover time for mud was much shorter in the controls than in the antibiotic treated systems.

2) By the rapid uptake of radiophosphate by water bacteria and their ability to hold the radiophosphorus from the chemical or colloidal adsorption mechanism of the mud, which would be accomplished by incorporating the phosphate into non-participating organic compounds. An affinity, or holding back by water bacteria of P^{32} would be indistinguishable from an accelerated return to the water from the mud.

Dead plankton deposited on the mud decay and greatly increase the removal of P^{32} from the water. This reaction also is blocked by antibiotics. In bottle experiments there is a natural fallout of bacterial cells of about 4% per day. Neither the redox state of the system nor the level of lake productivity could be shown to influence either living or inorganic exchanges. The events following addition of radiophosphorus can be described as a modified first order consecutive reaction in which PO_4 yields organic P in the bodies of bacteria which in turn yields organic soluble phosphorus to the water.

The rate of exchange is measured as turnover time, which is the time required for the appearance or disappearance of as much phosphorus as is present in the test material, say phytoplankton or water or mud. Some turnover times are: water of a whole lake, one week; water in a bottle over mud, 0.5 week; return from lake sediment in nature including rooted aquatics, 1 month; from mud in a bottle, 0.5 week; from bottle mud without bacteria, 2 weeks. Equilibration of PO_4 between water and the inside of bacterial or phytoplankton cells is almost immediate, say 5 minutes, but conversion to the organic state is slower with average turnover time 0.3 days. Rooted aquatics, probably cannot take up organic P; with inorganic P their time is 0.5 week. Zooplankton are opposite in behavior, unable to use phosphorus until bacteria have made it organic; their time is then 1 day.

INTRODUCTION

When phosphorus is added to a lake as fertilizer nearly all of it disappears in a few days (Smith 1945, Orr 1947, Pratt 1949). The decline, taken together with the known effects of fertilization of agricultural land, might be attributed to new growth, although no immediate algal blooms appeared. The theory of growth stimulation demanded reconsideration when it was discovered that tracer phosphorus atoms, measured as radio-

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activity, declined in the water in the same way as large masses of fertilizer (Hayes *et al.* 1952, Rigler 1956). It now appears that there is a single pool of phosphorus belonging to lake water and solids, which is distributed between them in a dynamic equilibrium or steady state. Disappearance from water is a consequence of an experimental arrangement in which phosphorus is added to the water phase. Were the opposite technique followed, of taking up phosphorus from the lake water on, say, ion-exchange resins, a continuous replacement from the solids would be expected.

The foregoing interpretation does not deny the general observation that addition of fertilizers to lakes stimulates growth. Obviously after equilibration there will be more nutrient in the system than before. The point is that the decline of phosphorus in the water phase is not a measure of increased productivity, since its rate will tend to be independent of the quantity added.

The oxygen relations of red blood cells in their plasma provide a simple analogy to the lake nutrient system. Oxygen is distributed between the phases in dynamic equilibrium. Under ordinary conditions nearly all the oxygen is in the cells. Any disturbance of either phase will cause an appropriate rearrangement of the system. For example, the addition of oxygen to the plasma would be followed by a "loss" to the cells and vice versa. To a physiologist the plasma, or fluid phase, is merely an innocuous transporter between the active parts of the system.

The dynamic equilibrium of a lake might be represented as

Phosphorus in aqueous phase, a small fraction of the whole	$\rightleftharpoons$	Phosphorus in solid phase, a large fraction of the whole.

with a constant value for each phase but with a continuous exchange between them. The radioactive tracer technique makes it possible to measure the rate of exchange, which is given as the turnover time, defined by convention as the time for as many atoms to move through the phase as are present in the phase. To illustrate turnover time, one might imagine a country of 16 million people (say Canada) in tourist equilibrium with a country of 160 million people (say U. S. A.). Suppose there are 32 million people crossing the border each way each year. The turnover time for Canada would be $^{16}/_{32}$ yrs or 6 months; for the U. S. it would be $^{160}/_{32}$ or 5 yrs. It will be noticed that there is no uptake of tourists by the U. S. although there would erroneously appear to be such if the only observation made were a count of persons leaving Canada. Also, it is not implied that every Canadian moves across twice a year. Many persons will not cross at all, others several times per year and still others will go over once and stay. Turnover time is not obtainable by counting the populations of the two countries, however frequently, for the counts do not change. It is necessary to detect persons actually crossing the border.

In limnological work the P^{32} is an identifiable sample moving one way, like Rotarians leaving Canada. Extra mathematics, over and above tourist-like counts, are made necessary by lack of knowledge of the total populations of phosphorus atoms in the two phases, so that indirect means must be used to secure turnover times.

The purpose of this paper is to discuss the effect on the exchange reaction of some natural variants in lakes, namely the level of productivity, the state of oxidation or reduction, and the presence of green plants and bacteria. In a lake all these will be acting together to produce the observed equilibrium, but in the laboratory the components can be separated.

An account of preliminary experiments is given by Hayes (1955), and the results to follow are an amplification and in one respect a modification of that report. Whereas the dominant role in exchange was previously attributed to the state of oxidation or reduction with bacteria playing a supplementary part, the results to follow indicate that bacteria are decisive and can to a considerable degree suppress the classical inorganic mechanism.

MATERIAL AND METHODS

Of the lakes whose sediments were studied, one was judged eutrophic (Southport Pond),

two were acid bog (Punchbowl and Silver), and five were unproductive or marginal (Copper, Black Brook, Grand, Lily, Bluff). For descriptions see Hayes and Anthony (1958).

Methods of handling and counting P^{32}, calculations of turnover times, handling of Jenkin sediment cores, etc. are given by Coffin *et al.* (1949), Hayes (1955), Hayes *et al.* (1952), and Harris (1957). Methods for measuring oxidation-reduction and for counting bacteria are given respectively in papers II and VI of this series.

In previous work (Hayes 1955) Jenkin core samples were collected to measure the radiophosphorus exchange between mud and water from lakes. This method is relatively exacting and inconvenient when many samples are required, and a simpler one was developed for the present investigation. Mud was collected in an Ekman dredge, the water was decanted off, and the surface mud was scooped into a sterile quart bottle. The remaining mud was put into a second sterile bottle. Lake water was also collected, and the samples were chilled and transported back to the laboratory as quickly as possible, generally within three or four hours. Here they were kept in the dark at 4°C until use, which was within a week or less. Before use the lake water was filtered through Whatman number 42 paper to remove large mud particles and plankton.

Artificial systems, as shown in Figure 1, were set up and maintained at 4°C. Before being used they were allowed to stand for five to seven days, to give the bacteria time to reach a stable number, under aerobic or anaerobic conditions, and to allow the whole system to reach biological and chemical equilibrium. When it was desired to inactivate bacteria the antibiotic terramycin, or later the more powerful tetracycline, was used. A quantity of 100 mg of terramycin was needed per bottle but only 15 mg of tetracycline. In practice 30 mg of the latter was used, to allow a safety factor. This concentration was found to inactivate the bacteria for at least a week. A further advantage of tetracycline was the absence of any observable deposit on the mud surface. The antibiotic was dissolved in a few ml of distilled water and then passed through a millipore filter to remove any resistant bacteria or moulds. It was added to the artificial system a day before the experiment was to begin. This interval was found to be sufficient to inactivate the bacterial population almost entirely.

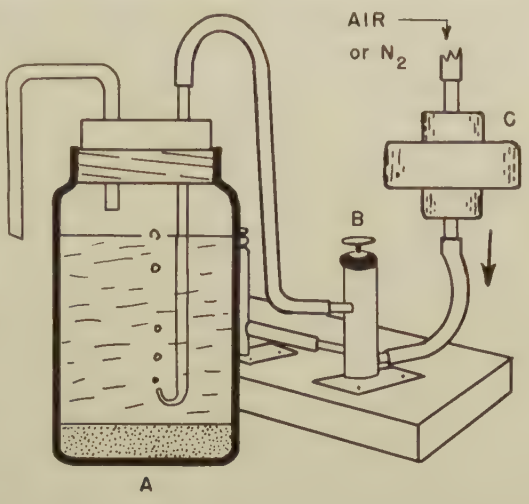

FIG. 1. An artificial mud-water system *A*, with gas jet connected to a needle valve *B*, and an aerosol bacterial filter *C*. Sterile apparatus was used and precautions against contamination taken so that the only opportunity for entrance of foreign bacteria was for about 15 sec. during collection of samples.

With a large syringe, about 20 ml of dredged mud was put into an 8-oz wide-mouthed bottle *A*, avoiding contamination of the sides. Twenty ml of surface mud was then added over the bottom mud and the bottles were capped and centrifuged for five minutes. The supernatant water was pipetted off, leaving a clean solid mud surface, over which, without disturbance, 150 ml of filtered lake water was added. Each bottle was closed with a rubber stopper through which passed a J-shaped jet with its tip turned upward and about 0.5 cm from the mud surface, and a U-shaped outlet tube. The jet was connected either to air or nitrogen which was allowed to flow at the rate of approximately one bubble per second, thus keeping the water in constant motion. The gas flow to each bottle was regulated by a needle valve *B*. The gas, air or nitrogen, was passed through an aerosol air filter *C* to remove any foreign bacteria.

In order to discover whether there was any appreciable uptake and exchange of phosphorus by bacteria on the glass surface of the bottle, water blanks were set up in each experiment without the addition of mud. There was no loss of P^{32} in the glass

blanks to which tetracycline was added. Further evidence of the effectiveness of tetracycline in inhibiting bacterial activity is seen in the investigation of qualitative changes in the radiophosphate added to Grand Lake and Punchbowl systems (see below). The culturing of water microorganisms by the millipore technique indicated that moulds are relatively unimportant numerically in lake water.

Attempts that were made to sterilize samples by heat under standard autoclaving conditions, were successful in killing all organisms, but produced such changes in the appearance and evident colloidal properties of the mud that they could not be taken as representative of natural conditions. Nevertheless, reactions with P^{32} agreed well with those of antibiotic sterilized samples.

Radiophosphate in dilute HCl, as obtained from Atomic Energy of Canada Ltd., was diluted with distilled water to give a count of 500,000 c.p.m. per ml and at once sterilized to kill bacteria and to permit storage for future use without the formation of organic radiophosphate by microorganisms. About one ml of the dilute radiophosphate was added to each artificial system, and duplicate samples were taken at intervals for counting.

From paper I, Table 2, the average total P in lakes is 18.5 ppb and the inorganic fraction amounts to 2.8 ppb. The added 500,000 counts in 150 ml water works out at 5.6×10^{-5} ppb. Thus the phosphorus increase in the samples by reason of added P^{32} amounted to 0.0003% of the total P already present or 0.002% of the inorganic P.

To separate the radiophosphate fractions a sample of water was passed through a millipore filter, which retained bacteria and particulate matter. The filtrate contained total phosphorus in solution, from which the inorganic P was precipitated. It was possible to find in a sample the c.p.m. per ml of radiophosphorus (a) in bacteria, (b) in particulate matter, (c) as inorganic P^{32} in solution, and (d) as total P^{32} in solution. The bacterial fraction could be estimated by comparing the counts in particulate matter with and without tetracycline treatment. There is a possibility that the small particulate fraction in the tetracycline-treated artificial systems might be incorporated in tetracycline-resistant bacteria or moulds, rather than in non-living particulate matter, although as already noted, moulds were not observed.

It was important to make sure that reduced conditions were secured in artificial systems by bubbling nitrogen through the water for a week. The cylinder nitrogen used was stated to contain not over 0.05% oxygen, which at equilibrium would give a dissolved oxygen level of 0.03 ppm. According to Mortimer (1941–2) ferrous iron was found in artificial systems below 0.1 ppm and in lakes below 0.5 ppm. Thus our oxygen level is theoretically low enough. Winkler tests on water through which nitrogen was bubbled were indistinguishable from zero oxygen.

Reduced conditions, however, are not attained merely by withdrawal of oxygen, but require the action of microorganisms, or of a suitable catalyst. The decisive test is the redox potential which, as paper II points out, is not easy to measure or interpret.

In an artificial Silver Lake oxidized mud-water system the initial potential was 0.33 volts for mud and the pH was 4.7. The water was then bubbled with nitrogen for four days, whereupon the potential of the mud fell to 0.14 volts. After bubbling with air for 12 hours, the mud potential rose to 0.40 volts. The same result was obtained with tetracycline treatment indicating probably that the antibiotic did not penetrate the mud to kill the bacteria there, but allowed them to reduce the system.

Further results on the Punchbowl are

TABLE 1. *Effect on potential of bubbling nitrogen or air in a Punchbowl artificial mud-water system*

The mud was initially reduced and the water oxidized. These results show that the mud-water system was well oxidized or reduced before the antibiotic was added and the experiments started.

Duration of bubbling in days	Potential in volts (Nitrogen)		Potential in volts (Air)	
	Water	Mud	Water	Mud
0	0.45	0.06	0.45	0.12
0.5	0.27	0.27		
3.0	0.17	0.14		
4.0			0.38	0.38

given in Table 1 showing that here also reduced conditions can be produced. With Grand Lake however, bubbling with nitrogen for over two weeks failed to bring about a corresponding reduction in four oxidized mud-water systems, to two of which antibiotic had been added. The mean initial potential of these systems was 0.42 volts for mud and 0.65 volts for water. The potential was measured every three or four days, the lowest reading after 15 days being 0.3 volts for mud and water. Other Grand Lake systems, when bubbled with air, remained oxidized at values between 0.4 and 0.5 volts for mud. It appears probable, in the light of these results and those in paper II, that the reduction achieved in these tests was as effective as would be found in nature in the sub-surface mud.

EXPERIMENTAL RESULTS

Natural and artificial cores compared

It was necessary for purposes of interpretation to know whether the mud-water radiophosphorus exchange in artificial systems represented the exchange under conditions more closely approaching those in the lake itself. The preparation of an artificial system involves the destruction of the natural physico-chemical and biological layering of the surface muds. If this layering were in any way peculiar and important to the phosphorus exchange in lakes, different results would be expected with natural Jenkin cores in which the mud-water interface is undisturbed. Two Jenkin cores were collected from Grand Lake and set up in the laboratory at 4°C. Water was siphoned off the cores until approximately 200 ml of water remained over the mud. Two artificial systems were set up by placing stirred bottom and surface mud in Jenkin cores and adding 200 ml of surface water over the mud in the usual manner. Tetracycline was added to the water of one natural and one artificial core. All cores were air bubbled.

The loss of P^{32} from the water is shown in Figure 2. It is apparent that natural and mocked-up Jenkin cores react similarly both in the presence and absence of bacteria. These results are almost identical to those for the eight-ounce bottle, artificial system

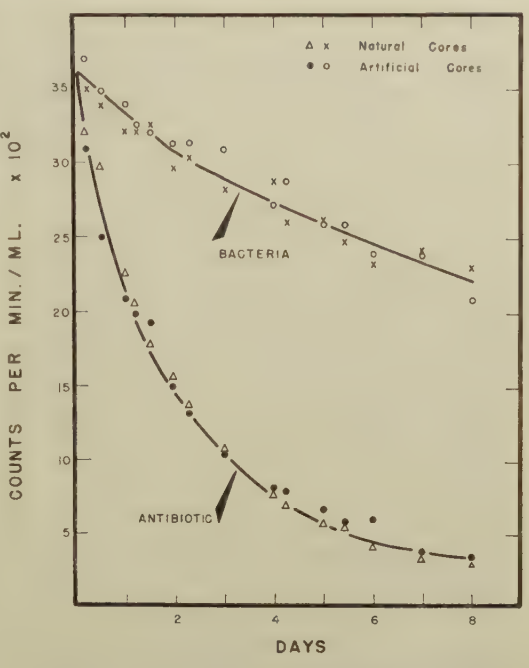

FIG. 2. The amount of radiophosphorus remaining in Grand Lake water over mud in natural and artificial Jenkin cores with and without antibiotic, *i.e.*, bacteria absent and present.

results with the same lake. Any heterogeneous structure of the natural mud-water interface, if it is essential, was obviously reestablished in the artificial systems before the P^{32} was added.

This experiment illustrates a general finding, namely that when bacteria are inactivated with antibiotic (or by heat sterilization) there is a rapid loss of P^{32} to the mud. With one exception, in more than 100 artificial mud-water systems tested the amount of P^{32} remaining in the water at equilibrium was greater in the controls in which bacteria were present than in the corresponding system to which antibiotic had been added. This was true whether the system was treated with nitrogen or air, *i.e.*, was aerobic or anaerobic. In the treated example under discussion, after a week less than 10% of the radiophosphate remained in the water. In the control two-thirds remained after the same period. This remarkable ability of bacteria to hold phosphorus in the water might be accomplished in two ways:

1) By an acceleration of the rate of P^{32}

return from the sediment to the water by bacteria in the mud. In all experiments, while the turnover time of water was generally of the same order of magnitude for all systems, the turnover time for mud was generally much shorter in the controls than in the antibiotic treated systems.

2) By the rapid uptake of radiophosphate by water bacteria and their ability to hold the radiophosphorus from the chemical or colloidal adsorption mechanism of the mud, which would be accomplished by incorporating the phosphate into non-participating organic compounds. An affinity, or holding back by water bacteria of P^{32} would be indistinguishable from an accelerated return to the water from the mud.

The marked atoms which were added follow along with the rest of the inorganic phosphate in solution. The test being in the dark, there is no green plant effect to consider, but only bacteria which are putting inorganic phosphate rapidly through their bodies, changing most of it enroute to the organic form. The organic fraction does not enter into non-living exchange reactions, of the kind described in earlier papers of this series. There is at the same time a regeneration of the inorganic phosphate by breakdown of the organic fraction, so that in the steady state of nature about 10 to 20% of the phosphorus in solution is inorganic.

When we prevent bacteria from entering the reaction by addition of antibiotic, the P^{32} remains in the inorganic state and is free to participate in, say, an adsorption equilibrium as discussed in paper III. This is the lower curve of Figure 2, showing the P^{32} atoms leaving the water. As each one leaves it is replaced by a conventional or P^{31} atom coming out of the mud, but these ordinary atoms have no identification marks.

A culture of phytoplankton

A side experiment with a culture of *Chlamydomonas dysosmos* illustrates the speed with which microorganisms can pass phosphorus through their bodies. It was a pure strain culture, bacteria free, and was well supplied, indeed over supplied, with nutrient in the medium so that no excess uptake due to possible starvation could occur. The phosphate concentration was 0.015% in the form of KH_2PO_4. The organisms made up 5% of the volume of the culture. Three-hundred counts per minute per ml of radiophosphorus was added to the medium, and its transfer from water to organisms followed, organisms being centrifuged from the medium before they were counted. In the final equilibrium the counts amounted to about 190 per minute per ml for the water and 1,760 counts per minute per ml for the cells. At the time of the first count, 2.5 minutes after the start of the experiment, equilibrium had already been established. On general grounds it is known that the turnover time for water is about one-third of the time for substantial completion of equilibration. Thus we can say that the turnover time for the phosphorus in the water was probably not more than 1 minute and may have been considerably less. At equilibrium the cells contained about half as much radiophosphorus as the water, and they had to return an atom to the water for every one they took out in exchange. It would thus appear that the turnover time for the phosphorus in *Chlamydomonas* cells must be not more than 0.5 minutes. Thus under natural conditions in a lake such microorganisms could very rapidly make over any inorganic phosphorus present into the organic form and return it to the water.

Competition between bacteria and plants

We turn now to the 8-oz bottle experiments, beginning with the competition for phosphorus between bacteria and higher plants. The mud layer at the bottom is omitted and the duplicates set up include a control of filtered lake water, the same plus one or other of the plants tested, and the same plus plant and antibiotic. Results on two lakes are shown in Figure 3.

The flowering plant *Eriocaulon*, which is very abundant over the bottom of Bluff Lake, was collected, and 1 g of sprigs was placed in each of two aerated bottles of Grand Lake water. The loss of P^{32} from the water is shown in Figure 3 at left. In the absence of bacteria *Eriocaulon* very rapidly takes up radiophosphate and reaches an equilibrium at which 10% of the P^{32} is left

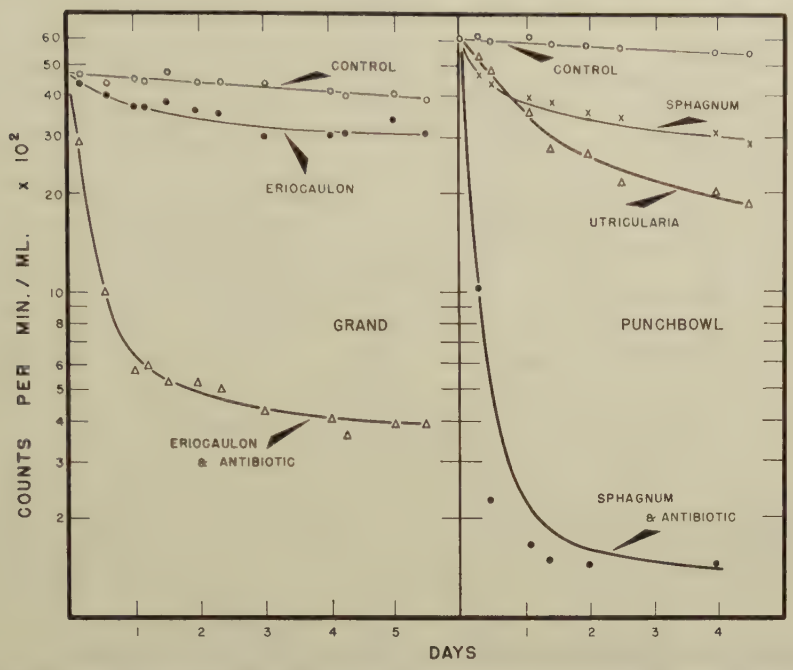

FIG. 3. Radiophosphorus remaining in aerated water (without added mud) from Grand Lake (primitive) and Punchbowl (acid bog) containing plants with and without antibiotic. The plants used were the pipewort (*Eriocaulon*), the bladderwort (*Utricularia*), and the peat moss (*Sphagnum*).

in the water. The phosphorus turnover times for *Eriocaulon* and water, in the absence of bacteria, were calculated to be 3.0 days and 0.34 days, respectively. The effect of bacteria is to hold phosphorus in the water and prevent the large loss to the bottom plants. When bacteria are present, the equilibrium between radiophosphorus in water and in mud was reached with two-thirds of the radiophosphorus in the water, including its bacterial population. This is because bacteria hold such large quantities of phosphorus in their cell bodies. A second cause is the production by the bacteria of soluble organic radiophosphorus, which the plant presumably cannot assimilate. This experiment was repeated with the plants *Sphagnum* and *Utricularia* in Punchbowl water, Figure 3 right. One and one-half grams of *Sphagnum* sprigs or 0.5 g of *Utricularia* sprigs were placed in 150 ml of water. Unfortunately tetracycline caused the *Utricularia* to turn brown, and there was little uptake by this plant in the presence of the antibiotic. Because of the adverse effect only the non-treated *Utricularia* result is plotted, which is similar to that for the other plants. In the absence of bacteria *Sphagnum* took up 97% of the radiophosphate in the water within half a day, the turnover times for *Sphagnum* and water being 3.5 days and 0.09 days, respectively. In the presence of bacteria 50% of the phosphorus remained in the water after 5 days. Although no turnover time for *Utricularia* and water in the absence of bacteria was obtained, the fact that the loss of radiophosphorus from the water in the presence of bacteria was greater for *Utricularia* than *Sphagnum* suggests that the former plant is as active as *Sphagnum* in exchanging phosphorus with water.

There was also a Grand Lake experiment including mud, to which *Eriocaulon* was added, and a Bluff Lake test including mud and *Utricularia*. This is placing all three components in competition. The two lake systems agreed well, that for Grand (Fig. 4A) showing that the addition of *Eriocaulon* to the control resulted in a loss of 70% of the radiophosphate from the water after six days. The ability of the plant to remove so

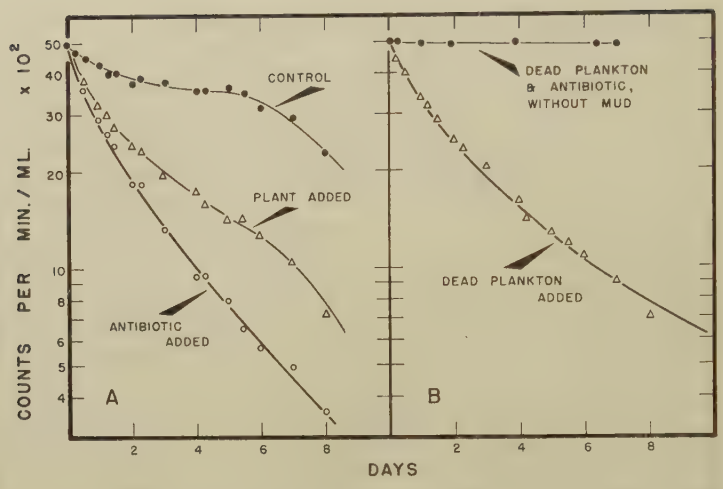

FIG. 4. *A*. Amount of P^{32} remaining in an aerated Grand Lake mud-water system to which 2 g of *Eriocaulon* sprigs or 30 mg of tetracycline was added. *B*. Effect of adding dead plankton in the presence and absence of bacteria. Comparing the lowest curve of *A* with the top one of *B* it is shown that mud is necessary for the inorganic exchange of P^{32} and that sterile plankton will not serve as a substitute for mud.

great a quantity of radiophosphate from the water when the mud control was unable to do so in the presence of bacteria indicates the affinity of *Eriocaulon* for phosphate. The control curve in Figure 4A is an extreme illustration of another phenomenon which was common to most experiments. While a control mud-water system seemed to reach equilibrium after three to eight days, a gradual loss of radiophosphorus continued over a period of weeks until practically none remained in the water. Evidence will be presented later which suggests this decline is due to a fall out of radiophosphorus in the bodies of bacteria. In the present example the turnover time would be calculated from the curve values before the sixth day.

In summary, when bacteria and higher plants compete for inorganic phosphorus the bacteria get there first and change a part of it to organic forms which are apparently unavailable to plant use. In the absence of bacteria the aquatic plants tested take up enormous quantities of P^{32} within 12 hr, indicating a rapid exchange and the establishment of an equilibrium with most of the phosphorus in the plant body.

Effect of plankton fallout

A large fallout of plankton following a bloom, might be expected to affect bacterial activity at the mud surface of a lake and thus alter the exchange of phosphorus. Plankton was netted from Grand Lake, autoclaved, and approximately 0.3 g was pipetted into duplicate mud-water systems from the same lake. The plankton sedimented immediately to the mud surface. Equal quantities of dead plankton were placed in two antibiotic treated glass blanks, omitting mud. Results are shown in Figure 4B. Obviously sterile dead plankton, of itself, is unable to adsorb significant quantities of phosphate by a colloidal mechanism or by other means. The addition of dead plankton to a mud-water system resulted in an enormous and rapid loss of P^{32} from the water. This greater uptake at the mud surface, as compared with the control in Figure 1A, was evidently caused by increased bacterial activity there, due to the large amount of readily decomposable organic matter. An uptake of phosphate by bacteria attached to falling organic matter in lakes is probably of importance in the natural cycle.

Effect of the state of oxidation

It is a matter of fact, repeatedly verified, that the removal of oxygen either over the lake bottom or in laboratory tubes will allow inorganic P to come up out of the mud. In

nature this will take place in the dark below the green plant zone. A large fraction though presumably not all of the new P remains inorganic. The conditions of stagnation which are prerequisite to oxygen lack also operate to keep the new P in a close layer a few cm thick over the bottom where it may reach a high concentration. Any increase which might be observed if the new P were dispersed through the whole lake, would depend on both the volume of the lake and the quantity of P produced. The latter measurement has hitherto been too difficult to make (as far as we know). The new P may formerly have been adsorbed on a gel which was destroyed when reduced, (paper III) or (as Einsele stresses) it may exist as a salt of which the reduced form (ferrous phosphate) is more soluble than the oxidized (ferric phosphate).

How would the above considerations affect the behavior of P^{32} in the water? We know that under ordinary aerobic conditions the loss to the mud is kept small through the intervention of bacteria. The effect of removing oxygen is unpredictable because of competition between two processes: (a) bacterial conversion to organic P will be if anything diminished, and this would enhance removal of P^{32} from the water (see Fig. 2), and (b) the general flow of ordinary P out of the mud would so affect the exchange mechanism as to leave most P^{32} in the anaerobic water. Turning to actual results, in four out of ten experiments, the per cent of radiophosphorus in the water at equilibrium was higher under aerobic than under anaerobic conditions. The average difference between the equilibrium values in these four experiments was 5.5%, the highest being 11.0%. These differences are so small that the two conditions may be said to be indistinguishable. In the six experiments where there was more radiophosphorus in the water under anaerobic conditions, the average difference was 20% with a range from 32% to 5%. It is concluded from the whole series that the activities of microorganisms are dominant, so that the inorganic mechanism cannot be identified.

When antibiotics are added to prevent bacterial synthesis one might hope to observe

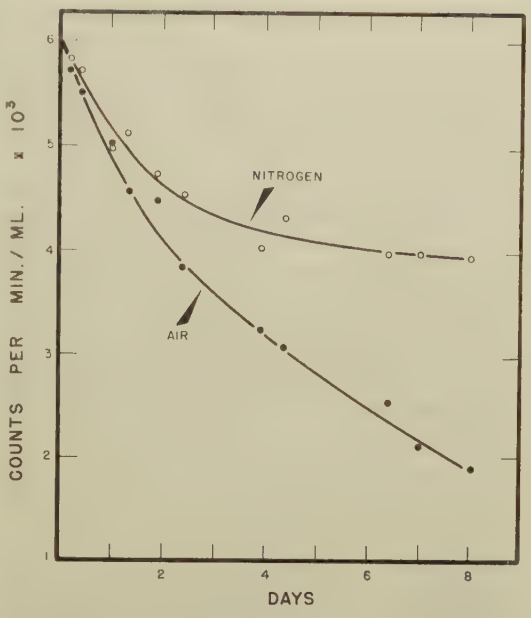

FIG. 5. The amount of radiophosphorus remaining in the water of antibiotic-treated Punchbowl mud-water systems under reducing (nitrogen) and oxidizing (air) conditions.

the inorganic mechanism, (b) of the previous paragraph. In ten out of twelve experiments the average per cent of radiophosphorus in the water at equilibrium was indeed higher under anaerobic conditions. In four out of these ten experiments however the difference was less than 5%. It was maximal in the two Punchbowl experiments in which there was twice as much radiophosphorus in anaerobic water at equilibrium (Fig. 5).

Effect of trophic level and lake type

Tests such as those described above have been carried out on eight lakes, with results expressed as turnover times for mud and for water and as P^{32} left at equilibrium. The data are in Table 2, in which the lakes are arranged in productivity groups. This judgment is in large measure qualitative, particularly as regards the separation between "marginal" and "unproductive."

A complete experiment would number 12 to 16 bottles each arranged as in Figure 1. Four artificial mud-water systems were bubbled with air and four with nitrogen. Antibiotic was added to two bottles in each

TABLE 2. *Turnover times for mud and water in 8-oz bottle experiments, and P^{32} concentration left at the equilibrium time*

The symbols are: A_c aerobic control; A_t aerobic test with tetracycline added to inactivate bacteria; N_c anaerobic or nitrogen control; N_t nitrogen with antibiotic added. Some heat sterilization results are also included, being placed for economy under the antibiotic values with which they are to be compared. The values are averages of duplicate tests or, in several instances, pairs of duplicates done at different times.

A mathematical limitation may be mentioned here, for illustration of which attention is directed to the upper curve of Figure 4. Suppose the flattening of this curve at 6 days and its subsequent decline were described by points with a little more scatter. The line marked "control" would then have to be drawn straight, a treatment which would make little difference to the turnover calculation for water, but would make the mud value read as infinity. The per cent left in water at equilibrium would come out to be zero. Such results, being meaningless, have been omitted from the table.

The treatment just described, involving the assumption that the decline, when plotted logarithmically, is linear, was the first way to calculate turnover time (Zilversmit *et al.* 1943). When applicable however, the complete method in which allowance is made for a two-way flow of phosphorus, yields more information.

Lake	Turnover time for mud Days				Turnover time for water Days				% P^{32} left in water at equilibrium			
	A_c	A_t	N_c	N_t	A_c	A_t	N_c	N_t	A_c	A_t	N_c	N_t
Productive												
Southport	3.2	13.8	2.1	8.6	3.8	3.0	1.7	2.1	53	17	44	20
Marginal												
Copper	3.4	11.3	2.5	22.0	2.0	1.7	3.7	2.0	36	13	65	9
Lily	4.4	26.5	5.0	10.7	1.9	3.3	2.0	2.5	31	10	28	19
ditto, heat sterilized				7.5		2.0		1.9		11		19
Black Brook	2.2	12.1	3.1	12.1	1.7	1.7	1.9	2.3	44	13	37	16
Unproductive												
Bluff	2.3	18.9	3.2	9.1	1.0	1.8	3.1	1.8	34	12	50	14
Grand	3.4	18.8	3.3	21.0	2.0	1.5	1.8	1.9	60	9	35	8
Acid Bog												
Punchbowl	6.3	7.0	1.6	3.7	3.8	3.1	2.7	4.1	41	30	62	56
ditto, heat sterilized		6.3		2.4		2.8		4.7		32		65
Silver			7.4		5.3	5.0	3.6	6.1			33	

group. In the Punchbowl and Lily Lake experiments heat-sterilized systems were also set up to compare with the effect of antibiotics. Each experimental arrangement was thus prepared in duplicate. Glass blanks (150 ml of water without mud) were set up under each condition in all experiments with the exception of Copper Lake and Bluff Lake. After the addition of P^{32} between eight and eighteen duplicate samples of water were taken from each bottle at intervals until equilibrium was reached or neared, and from these samples the turnover times for water and mud were calculated, as given in Table 2. They are averages of duplicate mud-water systems, the mean probable deviation of the two values corrected by the *t*-test being less than 20%. The per cent of the initial radiophosphorus concentration remaining in the water at equilibrium is also given.

The first conclusion to be drawn from Table 2 must be negative. Looking vertically at the columns, there is no clear relation between trophic level and the exchange reaction. Only the acid bog differs from the rest of the groups in the antibiotic or heat-treated "*t*" columns. Here the turnover time for mud is shorter and for water longer, and more P^{32} is left at equilibrium than in the others. Since the difference is in the sterile samples it is evidently related to the physical properties of the sediment rather than microorganisms. The brown water of bog lakes is carrying in and depositing large quantities of foreign organic material from surrounding swampy country which may account for the P^{32} effects.

The absence of differences in laboratory tests may not reflect behavior in nature, where phytoplankton enters the scene. These plants will not only compete with

bacteria for phosphorus, but may suppress them by their own antibiotics (Steemann Nielsen 1955).

Table 2 shows that the turnover time for mud, *i.e.*, the rate at which mud can put phosphorus back into the water, becomes greatly lengthened when microorganisms are inactivated. We may postulate a normal cycle in which there is (a) an uptake and synthesis of organic P^{32} in the water, followed by (b) a fall out to the sediment surface, where (c) a breakdown to inorganic P^{32} occurs under bacterial action, so that (d) the P^{32} is restored to the water. Under the tetracycline treatment an inorganic mechanism is in operation with different time relations.

Looking at the center block of Table 2, the horizontal values have no trend. Thus the turnover time for water is not shown to be affected by bacteria or by the state of oxidation-reduction.

The right block however, does indicate bacterial action, in that the columns A_c and N_c have higher values respectively than A_t and N_t. This means that when bacteria are present they hold extra phosphorus in the water. To look for an oxidation-reduction effect we compare column A_c with N_c and A_t with N_t. No convincing behavior pattern can be discerned, *i.e.*, there is no indication of more P^{32} left in the water under one state than the other. The one lake, Punchbowl, which clearly obeys theoretical demands, has already been illustrated in Figure 5.

No appreciable loss of radiophosphorus was noted from the water of over thirty glass blanks, both anaerobic and aerobic, to which antibiotic had been added. This is evidence that the loss from the water of mud-water systems was not due to precipitation of phosphate as ferric phosphate.

The agreement between heat sterilization and antibiotic treatment of mud-water systems suggests that: (a) the effect of antibiotic on P^{32} exchange is due to the inactivation of bacteria; (b) moulds and other antibiotic-resistant microorganisms are relatively unimportant in the mud-water exchange; (c) the physico-chemical adsorption mechanism of the mud is not drastically affected by the very harsh treatment of heat sterilization.

A look at the whole of Table 2 suggests that the action of bacteria is to suppress the underlying inorganic exchange mechanism, which is thereby relegated to a subsidiary role. We may suppose that in nature the phytoplankton behaves in the same way.

Loss from the water of control glass blanks

There was a loss of radiophosphate from the water of over thirty control blanks, that is, blanks in which the bacteria had not been killed. This loss was generally much less and occurred at a slower rate than that from corresponding mud-water systems.

Some blanks showed an equilibrium with a solid phase, evidently periphytic or sedimented organisms. In most cases the decline was arithmetic during the time of sampling. Often there was no decline during the first few days followed by slow loss of radiophosphorus. Full data dealing with these results are not presented here because of their voluminous and diverse nature. The loss of radiophosphorus from Grand Lake and Punchbowl glass blanks is shown in Figure 3.

Heukelekian and Heller (1940) report that the bacterial population of stream water is increased by addition of clean sand, while ZoBell (1943) found that increased bacterial activity in stored sea water is directly proportional to the ratio of glass area to water volume. According to Taylor and Collins (1949) growth is stimulated by Bohemian glass but not by Pyrex or fused silica. These reports led us to enquire whether the loss of P^{32} in the control glass blanks was due to bacteria on the sides of the glass bottles. If this were so it would be a source of error in the mud-water systems. If however, the loss were due to fallout of bacteria from the water to the bottom of the bottle, or to accumulation by sessile bacteria on the bottom, there would be no error introduced.

Tests were conducted on Punchbowl mud-water systems in which the water volume was reduced from the usual 150 ml to 100 ml and 50 ml, and to which pieces of glass tubing were added to double the glass surface. Tabular data will be omitted since all

results were negative, *i.e.*, there was no difference in behavior of P^{32} caused by changed surface-volume ratios.

It is known (Fig. 3, lines marked "control") that some loss occurs from water even when mud is not present. To discover whether such loss of radiophosphorus might be due to a fallout of bacteria, a glass filter disc was placed on the bottom of an aerobic Grand Lake preparation. After 15 days 50% of the radiophosphorus remained in the water. The sintered glass disc, when it was removed and its activity tested, accounted for 89% of the P^{32} which had disappeared from the water. Thus most of the loss of P^{32} is to the bottom of the bottles, and the effect is not a source of error where the mud surface replaces the glass bottom of the blank. It is concluded that periphytic bacteria, if they are present on the glass walls, are not active in taking up radiophosphorus.

Consecutive forms of phosphorus

It has already been suggested that tracer phosphate is soon taken up by bacteria and changed into the organic state, and that later some of it leaks out into the water again as soluble organic phosphate. We may now consider the time and equilibrium relations of the process which may be compared and contrasted with a consecutive reaction, $A \rightarrow B \rightarrow C$, as shown in the left block of Figure 6, in which the two reactions are proceeding simultaneously. If events were followed by analyzing for A, curve A would be obtained; if periodic measurements were made of the end product C, curve C would result; finally if only the intermediate product B were determined, the course of the reaction would rise to a maximum and fall off as shown by curve B.

The course of events with a P^{32} experiment differs from the reaction described in that there is a gradual loss of the total, A + B + C, from the water both by reason of fallout, etc. as already described, and by inorganic exchange with mud if mud is present. Also the lake water reaction does not proceed to virtual completion, but to an equilibrium in which appreciable quantities of all forms remain. As already mentioned general water analyses show 10 to 20% of the phosphate as inorganic, *i.e.*, corresponding to A.

Three aerated bottle series were set up, with and without mud, in which the P^{32} in several fractions was followed. Two were on Grand Lake material, collected in January and July and the third on Punchbowl collections made in July. Results agreed very well, and the Punchbowl, for illustra-

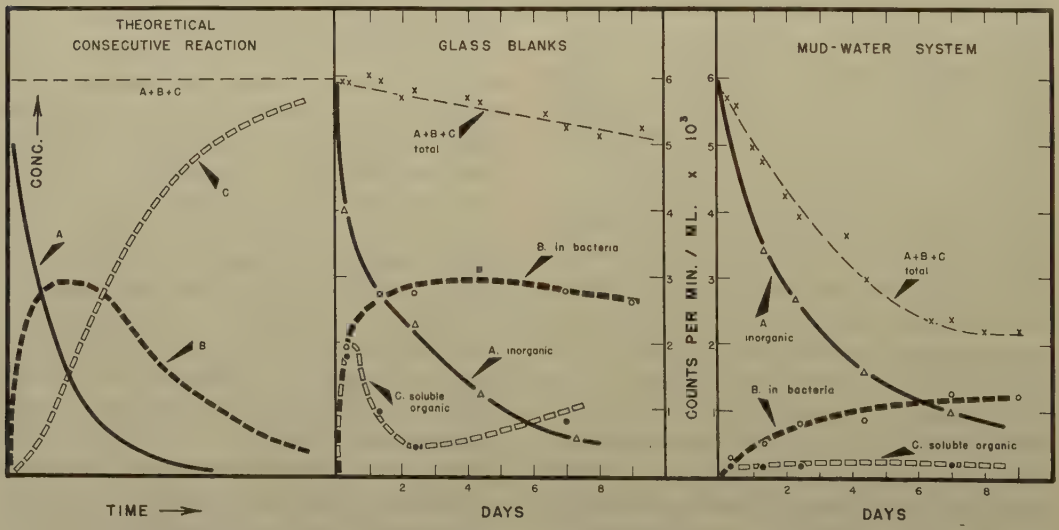

FIG. 6. *Left:* Pattern of a consecutive first-order reaction of type A→B→C. The second step is half as fast as the first. From Getman and Daniels 1937, p. 331. *Center:* Forms of P^{32} as they vary with time in an aerated bottle of Punchbowl water. *Right:* The same in a mud-water system, set up as shown in Figure 1.

tion, will be presented in detail, as shown in Figure 6.

Rapid changes were observed in the P^{32} which had been introduced as inorganic PO_4. Figure 6, center, shows the water blank for the first nine days. Water bacteria rapidly incorporated 50% of the P^{32} as part of their body protoplasm, and reached virtual equilibrium within 12 hours. (The same equilibrium at 12 hours was observed in a test on Black Brook water by which time two-thirds of the P^{32} was in the bacteria.)

There was a rapid formation of soluble organic radiophosphorus (C) reaching a maximum by the time of the first observation at twelve hours. This fraction then fell to an equilibrium value after two days. The maximum might be due to a very rapid production via bacteria and a slower loss from the water to mud or sessile microorganisms. The subsequent change in C was slight with a possible rise at right. While the increase at 7 days is not proven by the single analysis, it is made plausible by the theoretical necessity that the total A + B + C should sum up to the value for the top line. The turnover time for the bacteria was calculated at 5 hr.

When mud is added to the system (Fig. 6, right) the uptake of P^{32} by water bacteria is considerably diminished. The reason is apparent in the difference between the topmost or "total" curves showing the great loss from the water when mud is present. In other words there is introduced with the mud a great number of competing bacteria as well as an inorganic exchange mechanism. The soluble organic fraction (C) in the water is also smaller when mud is present. It appears that the two experiments are showing the same phenomena but that the mud one is modified by the steep down slope of the A + B + C curve. The turnover time for water bacteria worked out at four days, nearly an order of magnitude larger than when mud is absent. Evidently the mud is pulling for P^{32} against the water bacteria, which tend, as a consequence, to hold on to what they can get.

In Grand Lake tests the mud has diminished effectiveness as a P^{32} remover (see Fig. 4A, top line). For this reason the turnover time for water bacteria was about the same as that of the Punchbowl water blank, 4 hr or less. As a further consequence there was a greater uptake by the water bacteria (B). In other words the Grand Lake complete system was not unlike the Punchbowl water blank in behaviour.

INTEGRATION

There are now sufficient data, as assembled in Table 3, to warrant a preliminary assessment of the time relations of the several phosphorus reactions.

For the water of a whole lake, the turnover time averages about a week (center col., blocks A and B). For a bottle experiment it is nearly half a week (block C, 1st 4 entries). The bottle experiments lack phytoplankton and rooted aquatics. The two results are surprisingly alike, and they seem at first to contradict the tourist analogy of the introduction, according to which a lake of infinite depth (compared to a bottle) would have an infinite population of phosphorus atoms, hence an infinitely long turnover time. The fact seems rather to be that in these lakes the exchanging mechanism builds up with the water volume, perhaps because the littoral zone is large and phytoplankton is proportional to volume of water. In our analogy the tourists are proportional to the population of the country.

The return from solids (left col.) is an order of magnitude slower in lakes than in bottles, presumably because of extra competition from rooted aquatics, phytoplankton, and zooplankton in the natural state. We suggest provisional acceptance of the bottle results as describing the behavior of mud alone. The lack of difference between oxidized and reduced systems and the four-fold slowing of the response when bacteria are inactivated has already been discussed.

The remainder of Table 3 deals with reactions between aquatic life and water in absence of mud. With microorganisms a sharp time distinction exists between two processes. There is a quick reaction, evidence for which rests mainly on Rigler's results with freshly collected lake water in-

TABLE 3. *A collection of turnover times*

The first three lake experiments are with large quantities of phosphate as fertilizer; all the others are with tracer amounts of radiophosphorus. Although added as inorganic PO_4 the phosphorus is soon changed to organic compounds when bacteria are present. Times are given in days except for equilibria between outside water and cells, which are given in minutes (shown by italics in table).

Turnover time for 1st mentioned component	Equilibrium system	Turnover time for 2nd mentioned component	Lake, location, experiment	Reference
	A. Whole lakes. Addition of dry phosphate fertilizer			
176	Solids, *i.e.*, mud + plants, *vs.* water incl. bacteria	17	Unstrat. Crecy, N. B.	Smith 1945
	Solids, *i.e.*, mud + plants, *vs.* water incl. bacteria	3.2	Unstrat. marine Loch Craiglin, Scotland	Orr 1947
	Solids, *i.e.*, mud + plants, *vs.* water incl. bacteria	2.4	Unstrat. marine pond, Cohasset, Mass.	Pratt 1949
	B. Whole lakes. P^{32} tracer experiment			
37	Solids, *i.e.*, mud + plants, *vs.* water incl. bacteria	7.6	Punchbowl, N. S. epilimnion	Coffin *et al.* 1949
39	Solids, *i.e.*, mud + plants, *vs.* water incl. bacteria	5.4	Unstrat. Bluff, N. S.	Hayes *et al.* 1952
	Solids, *i.e.*, mud + plants, *vs.* water incl. bacteria	3.6	Toussaint, Ont. epilimnion	Rigler 1956
29	Solids, *i.e.*, mud + plants, *vs.* water incl. bacteria	10.2	Toussaint, recalculated by us from Rigler data.	
	C. Laboratory experiments with P^{32}			
3.6	Mud *vs.* water, with bacteria present, oxidized system	2.7	Average of values in Table 2	Orig.
3.5	Same, but reduced system	2.6	Average of values in Table 2	Orig.
15.5	Mud *vs.* water, with bacteria absent, oxidized system	2.6	Average of values in Table 2	Orig.
12.5	Same, but reduced system	2.9	Average of values in Table 2	Orig.
1.1	Inorg. phosphorus in sol. & in bact. *vs.* org. phosphorus in sol. & in bact.	0.79	Filtered surf. water from Chocolate, a polluted lake near Halifax	Harris 1957
0.21	Inorg. phosphorus in sol. & in bact. *vs.* org. phosphorus in sol. & in bact.	0.21	Same from Punchbowl N. S.	Orig.
0.2	Inorg. phosphorus in sol. & in bact. *vs.* org. phosphorus in sol. & in bact.	0.2	Same from Grand, N. S.	Orig.
	Bacteria & algal cells *vs.* water	*4.5*	Surf. water freshly collected from Toussaint, Ontario	Rigler 1956
	Bacteria & algal cells *vs.* water	*3.6*	Same from Oiseau, Ont.	Rigler 1956
	Bacteria & algal cells *vs.* water	*2.6*	Same from Maskinonge, Ont.	Rigler 1956
0.5	Phytoplankton culture *vs.* water	*1.0*	Pure *Chlamodomonas* in laboratory nutrient solution	Orig.
3.0	Flowering plant *Eriocaulon vs.* water	0.34	1 g sprigs in 150 ml lake water; bacteria absent	Orig.
3.5	Peat moss (*Sphagnum*) *vs.* water	0.09	1.5 g sprigs in 150 ml lake water; bacteria absent	Orig.
0.58	Brine shrimp (*Artemia*) *vs.* sea water		Naturally occurring bacteria present	Harris 1957
∞	Same, but bacteria absent		P^{32} remains in inorganic state	Harris 1957
1.8	Beach flea (*Gammarus*) *vs.* sea water		Naturally occurring bacteria present	Harris 1957
∞	Same, but bacteria absent		P^{32} remains in inorganic state	Harris 1957

cluding natural phytoplankton, and presumably some bacteria; there is also a slow reaction observed in our laboratory results with bacteria but not including phytoplankton. These are resolved below (Fig. 7) into a harmonious scheme, for the validity of which further proof is desirable. In the scheme it is supposed that PO_4 in water goes in and out of the bodies of phytoplankton and bacteria with a turnover time of the order of 5 min. Most of it is chemically unchanged. (One is tempted to think about the way glucose or urea crosses cell walls in a higher animal.) The process of conversion to the organic form is about two orders of magnitude slower, with a turnover time averaging 0.3 days.

Two plants tested had the same turnover times as natural mud. As previously noted they were apparently unable to take up organic phosphorus, although we have not yet proved this. Proof would consist in offering plants organic P^{32} in solution with no inorganic fraction present.

Finally, two marine invertebrates are reported which may be considered to indicate the expected behavior of zooplankton. They are unable to take up inorganic phosphorus, but when bacteria are present to serve as food, their turnover time is of the order of one day.

In Figure 7 the data on turnover are combined in a single plan. The numbers are all days except for one 5-min entry. Four kinds of line are used as qualitative indicators of time. It is seen that upon addition of phosphate to water the immediate reaction, within minutes, is a transfer through the bodies of unicellular floating forms of life (heavy lines to right). Next, as shown by the light solid lines to the left, there occurs within a matter of hours, *i.e.*, two orders of magnitude slower than the above, an exchange in which the floating cells and the higher aquatics compete on approximately equal terms for the PO_4. This they make into their own body structures, throwing some of it back to the water as soluble organic phosphorus. We have cancelled out the virtually instantaneous passage through floating cells and set the process down as an equilibrium between

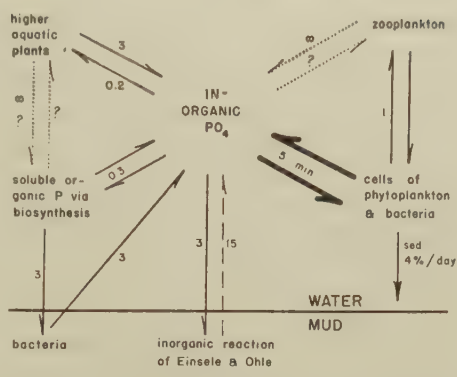

FIG. 7. Transformations of phosphorus in a lake with turnover times for different equilibria. Very heavy lines indicate the first reaction with floating cells, time in minutes. Other times in days. Lighter solid lines are reactions at intermediate speeds—two or three orders of magnitude slower than the initial one. Dashed line is the return from mud by inorganic release, a still slower turnover. Dotted lines at top indicate reactions too slow to measure, called infinitely slow by comparison with the rest.

inorganic and organic phosphorus in solution, for which the turnover time is 0.3 days.

At top left is indicated a doubt as to whether higher plants can utilize organic phosphorus, and at top right the feeding of zooplankton is given a turnover time, and their inability to utilize inorganic P is noted.

The lower part of Figure 7 brings in the sediment surface. At right bacteria are noted to fall out at a few per cent per day. This is in bottles and not to be read as net fallout in lakes, which is subject to wide variation. The fallout at right probably describes the same phenomenon as the line leading down at left, *i.e.*, the settling of organic matter to be reduced again by bottom microorganisms for regeneration to the water. The turnover time for leaving the water and for return is here 3 days, an order of magnitude slower than for exchanges with floating life.

The inorganic mechanism, which can be observed when bacteria are suppressed, is shown at lower center. There are variants for reduced and for oxidized conditions which have been described in papers II and III of this series. As already noted, the observed time relations are not affected by the redox state. In taking out inorganic P

the mechanism has the same turnover as the bacterial action just described, namely 3 days. The return to water however is much slower, at 15 days. This means that there is about $15/3 = 5$ times as much participating inorganic P in the mud phase as in the water (as in our tourist example the U. S. has a larger population than Canada). Now as previously noted, the ratio of soluble organic to inorganic P in the water is also 5 to 1. Putting the ratios together we conclude that the P exchanging in the mud by the inorganic mechanism is about equal to the total P in the water. Thus if we could get it all released it would double the lake supply. (However the removal of the participating mud pool would trigger a further equilibration reaction by which more phosphorus in the mud would become available and so on *ad infinitum*, like the flow of ink out of a fountain pen.)

The final level of total exchangeable phosphorus in a lake is eventually determined by: (1) The net rate at which phosphorus in solution and in particulate matter enters the lake through drainage and rainfall. Ruttner (1953) states that the spring waters entering Lunz Lake contain 0 to 3γ/L of phosphate, while rain water occasionally contains 10γ/L of phosphorus originating in dust. This inflow is dependent on the geology, fertility, and size of the drainage area, and on the amount of rainfall. (2) The rate at which exchanging phosphorus is lost from further metabolism by incorporation into inorganic insoluble precipitates and undecayed organic matter in the lake bottom. Phosphorus in sediments amounts to about 0.05% dry wt of which two-thirds is organic. The largest concentrations and highest organic fractions are in surface sediments of recent geological formations (Kleerekoper 1957). (3) The morphometric properties of a lake. The volume determines the extent to which phosphorus is diluted. The area and depth influence the thermal stratification and relative size of the photosynthetic zone, which in turn affect the phosphorus cycle.

Within the limits set by these properties the various parts of the phosphorus cycle will operate. For example rooted aquatics are probably unimportant in large deep lakes where the littoral region is negligible compared to the total volume. In smaller and shallower lakes, in which the profundal zone is minimal, the growth and bacterial decay of rooted aquatics probably dominates the phosphorus exchange. It is even possible for the whole productive capacity to be occupied by rooted aquatics, leaving no nutrients to spare for support of phytoplankton (Prowse 1955).

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A Submersible Sampling Pump[1]

The Chesapeake Bay Institute now has in operation a sampling pump which is completely submersible in water of depths in excess of 200 feet. This pump, which is a product of the combined efforts of Chesapeake Bay Institute personnel, is in our estimation the solution to two critical problems associated with the commonly used water samplers in estuarine research today. The first of these is to obtain a water sample of sufficient volume to enable the investigator to perform the many chemical, physical, and biological analyses that are often necessary to make a complete survey of an area. The second is that this ample supply of water be one which is unaltered in dissolved gas content so that valid dissolved oxygen determinations can be made.

The stock type water bottles appear to satisfy neither of these. They obtain, in most instances, only a liter of water at each lowering and yield samples of varying dissolved oxygen content from a series of replicate lowerings. A deck pump supplies a sufficient amount of water, but the formation of bubbles in the line alters the dissolved gas content.

In situ measurements are ideal, but often require elaborate instrumentation and involve the lowering of many of these instruments over the side to obtain all the necessary data. With a continuous, confined flow of bubble-free water passing through the laboratory it is possible to incorporate instruments in the line and eliminate pressurized casings and water-tight fittings. For example, the Chesapeake Bay Institute has a continuous-recording pH meter which can be connected to the hose line, and we are at present designing a modified induction conductivity temperature indicator, an oxygen electrode, and a hydrophotometer for the same type of operation. Hence, it will be possible by lowering a single sampler over the side to obtain a great many measurements in a short period of time.

The unit (Fig. 1) consists of a centrifugal pump coupled to a submersible electric motor of the type used in submersible deep well pumps. These are mounted in a brass frame which can be suspended from a B.T. wire. The pump has a ¾-inch intake and a ½-inch outlet and is rated at 21 gallons per minute with no head. However, with 200 feet of ⅝-inch I.D. garden hose and approximately 5 feet of head (the distance from the water surface to highest level reached by the hose), the unit delivers from 3 to 4 gallons per minute. The loss in efficiency is due principally to wall friction in the hose. A double set of cylindrical wire screens was placed over the intake of the pump to exclude large pieces of detritus and sea nettles.

The motor is a 115-volt AC, ⅓-horsepower, water-filled, and water-cooled unit obtained from the Franklin Electric Company in Bluffton, Indiana. The starting and running controls are contained in a separate box which is mounted in the laboratory. The motor jacket is of stainless steel with cast-iron upper and lower bearing housings which are coated with a metal preservative. The motor is arranged so that in case of loss of liquid from the inside for any reason, such as seal leakage, additional water will seep through a filter in the top casting to make up for the loss. The windings inside the motor are isolated from the water, and there is only the possibility in the event of sea water seepage of slow corrosion of the rotor which rotates in the liquid.

A field test for dissolved oxygen was conducted in which samples were drawn from the pump line and collected simultaneously with two types of water bottles lowered to the same depth as the pump. An oxygen depleted layer was chosen since it was felt that the only possible cause for error would be a leakage of air into the hose line between the pump and the bottle into which the sample was being drawn. The results of this test are presented in Table 1.

It will be noted that one of the determinations from each water bottle is 30% to 40% higher than the other determinations. At this oxygen level this is a common occur-

[1] Contribution No. 38 from the Chesapeake Bay Institute. This investigation was supported by the Biology Branch of the Biological Sciences Division of the Office of Naval Research.

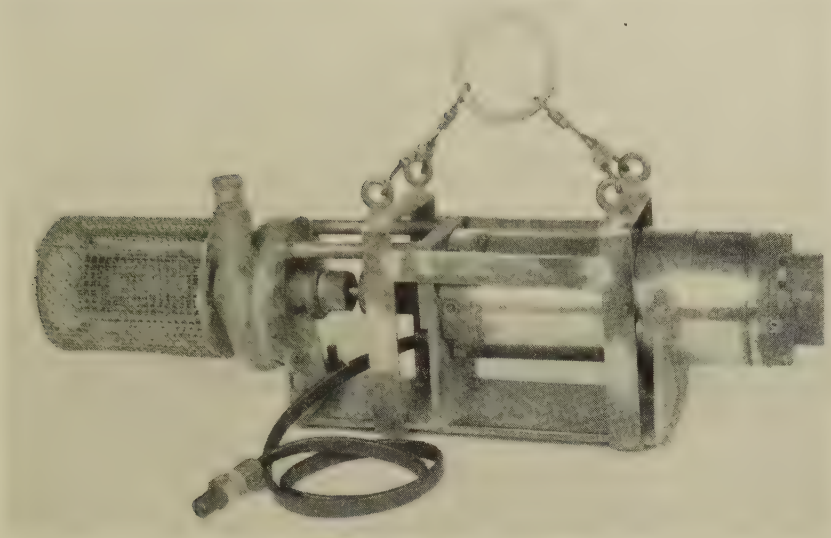

FIG. 1. Submersible sampling pump assembly: *right*, water-filled electric motor; *left*, centrifugal pump, the intake of which is protected by a double screen.

TABLE 1. *Dissolved oxygen in milliliters per liter of samples taken simultaneously by three types of samplers*

Sampling was done in 42 feet of water at slack tide.

Sample No.	Submersible pump	Kemmerer bottle	CBI snapper bottle
1	0.71	—	0.73
2	0.71	0.75	0.94
3	0.73	0.78	0.74
4	0.75	0.95	0.79
5	0.71	0.75	0.79

TABLE 2. *Pumped samples of dissolved oxygen in ml/L versus siphoned tank samples*

Pump	Tank
5.48	5.48
5.48	5.48
5.47	5.48
5.47	5.48
5.23	5.22
5.23	5.23
4.47	4.47
4.48	4.47
4.04	4.03
4.04	4.03
4.01	3.99
4.01	3.99
2.97	2.98
2.98	2.96
1.15	1.17
1.15	1.15
1.01	1.01
1.01	1.01
0.91	0.92
0.92	0.92

rence and is undoubtedly due to air leakage, either into the D.O. bottles as the sample is being drawn, or into the sampling bottle itself. This type of result is even more prevalent with slow draining water bottles. Here there seems little probability of obtaining consistent dissolved oxygen determinations at low concentrations, since there is a greater opportunity of exchange with the atmosphere. The D.O. bottles are filled very rapidly from a ¼ inch "T" fitting in the hose line of the submersible pump.

Since all of the values obtained with the submersible pump were below those obtained with either water bottle, it was felt that additional data were needed to establish the pump as a sampler of dissolved oxygen. Therefore the pump, with 100 feet of hose attached, was placed in a constant temperature water bath and duplicate samples were drawn from the hose line simultaneously with samples siphoned from the tank. The dissolved oxygen content of the water was decreased by bubbling nitrogen gas through it. The results of these tests (Table 2) clearly demonstrate the ability of the pump to obtain a valid dissolved oxygen sample.

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An Observation on the Spawning of the Sea Scallop, *Placopecten magellanicus* (Gmelin), on Georges Bank

The spawning of the sea scallop, *Placopecten magellanicus* (Gmelin), was observed in late September in the natural habitat of the scallop on Georges Bank at 41° 15′ N. Lat. and 66° 22′ W. Long. The northeastern part of Georges Bank is the most productive and most intensely fished sea scallop ground on the Atlantic coast. Between 1944 and 1956, 77 million pounds of sea scallop meats (adductor muscles only) representing about 2.5 billion individuals were taken from these beds.

From September 21 to 25, 1956, an average of 250 individuals was examined each day. None of those examined on September 21 had begun to spawn, but spawning began during that night. The smaller scallops, ranging in size from 50 to 90 mm, began spawning first and were nearly spent when most of the scallops larger than 90 mm began spawning. Eighteen hours later, on September 22, 48 per cent were either spawning or already spent. At the close of the observation period on September 25, 92 per cent were either spawning or already spent.

All the sea scallops examined later at other locations on the bank were spent. Bottom temperatures were between 8° and 11°C. The trigger mechanism of the spawning is not known.

In many instances, eggs being released by a spawning female had adhered along the margin of its upper, rounded valve. Although these adherent eggs were not positively identified, they were assumed to be scallop. In all these instances, the eggs on the valve and within the gonad were the same size and color.

The condition of the gonad in the sea scallop during spawning proceeds through three distinctly different stages which can be observed macroscopically. A ripe male gonad is firm and plump with a milky-white color. When spawning begins, this coloration becomes invaded with irregular, grayish, clear channels. These clear channels gradually engulf the entire gonad. When completely spent, the gonad is collapsed and is clear or translucent. A ripe female gonad is a brilliant coral red. As spawning begins, the color fades to a pale pink and the eggs seem to be somewhat larger. Clear channels appear and invade the entire gonad as in the males. At this stage, nearly spent, the sexes become difficult to distinguish without close examination.

The time of spawning observed here closely agrees with the late September and early October period mentioned by Posgay (1950) for Cape Cod Bay. The period of spawning, however, is considerably shorter than that reported for the Digby beds by Dickie (1955).

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Influence of Oxygen Tension on Respiration of Phytoplankton

The Winkler method of determining oxygen is often used to measure the rate of photosynthesis of freshwater and marine phytoplankton communities. In order to find the actual rate of carbon assimilation, the amount of oxygen lost in the dark bottle is added to the amount of oxygen produced in the light bottle.

Many investigations have been carried out regarding the question whether or not respiration is influenced by light. In the light-dark bottle method of Gran men-

tioned above it is assumed that respiration in the dark bottle is equal to or not much different from respiration in the light. However, not only illumination but also the amount of oxygen available to the respiring cell may be of importance. During photosynthesis respiration takes place in a very high tension of oxygen liberated by photosynthesis, although not in pure oxygen. The effect described in this paper, however, is not the same as photooxidation, since it occurred in dark bottles.

It may be asked, therefore, if oxygen tension influences respiration. To answer this question measurements of respiration were made by the Winkler method for

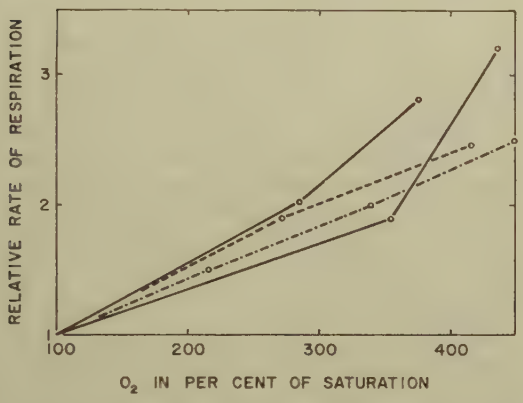

FIG. 1. Respiration rates of natural diatom communities. Four series in supersaturated water, showing the increase of O_2 uptake with increasing O_2 tension.

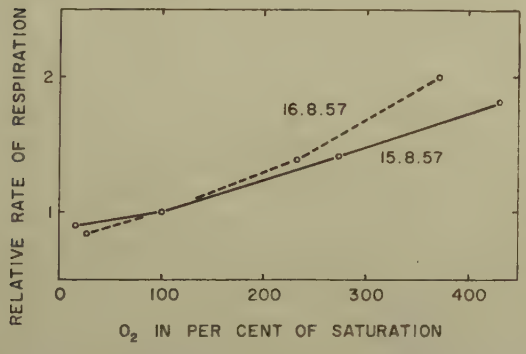

FIG. 2. Respiration rates of a natural *Anabaena* community in under- and supersaturated water. Two series of experiments on successive days. Each point on the curves is the average of 10 single determinations.

TABLE 1. *Respiration over a 5-hour period for an Anabaena community at various saturation levels of oxygen*

Each value is the mean of 10 determinations.

Saturation level of O_2 %	Respiration mg O_2/L
100	2.584 ± 0.11
230	3.560 ± 0.16
373	5.088 ± 0.24

several natural limnic phytoplankton communities in water super- and undersaturated with oxygen. Replicate samples of natural diatom (*Synedra acus delicatissima* and *Asterionella formosa*) and *Anabaena* (*A. flos aquae*, *A. spiroides*, and *A. inaequalis*) phytoplankton communities were placed in 250-ml glass-stoppered bottles and allowed to remain in the dark at constant temperature after first having been brought to a known level of oxygen supersaturation. Control experiments with water that had been ultrafiltered to remove all microorganisms showed no change in oxygen content over several days.

In the experimental bottles the rate of respiration increased as the per cent saturation of the water was increased, as shown in Figures 1 and 2. Note in Figure 2 that the relationship between rate of respiration and per cent saturation apparently holds for undersaturated water as well. The rates of respiration in the figures are reported in relative terms, using the respiration at 100 per cent saturation as equal to unity. This was necessary because no attempt was made to relate respiration to number of cells, volume of cells, surface area, etc. Absolute values for the *Anabaena* series of 16 August 1957 are given in Table 1 as an example. Thus at roughly 400 per cent saturation, respiration in *Anabaena* was about twice as great as at 100 per cent.

This considerable influence of oxygen tension on respiration is surprising, because it is known that an O_2 tension of 10^{-4} atm. is sufficient for oxidation by the enzymes of the cell. We must suppose, therefore, that the rate of diffusion is the limiting factor even in unicellular organisms the size of phytoplankton and not only in the big

thalli of marine algae and the bodies of higher aquatic plants (Gessner and Pannier 1958).

This interpretation is substantiated by results from the Warburg method. The same *Anabaena* population in Warburg vessels shaken 100 times a minute for 20 minutes showed *no* difference in respiration between samples kept under 100 and 400 per cent oxygen.[1] Thus, with shaking, an oxygen tension of 100 per cent (water in equilibrium with air) is sufficient for maximal respiration. The same result was obtained by Franck and French (1941) with round pieces of *Hydrangea* leaves. Using the Warburg method they found 20 per cent of oxygen to be enough for maximal respiration. Between 20 and 100 per cent saturation no further increase of oxygen uptake could be demonstrated.

On the other hand, in the present experiments when the *Anabaena* flasks were *not* shaken during the experiments but only briefly at the end to establish an equilibrium between the gaseous and water phases, the Warburg manometers showed the same differences in respiration between 100 and 500 per cent saturation as had been found with the Winkler method. These differences disappeared only in *strong* water movement. Bottles rotated slowly in a klinostat gave the same differences by the Winkler method as stationary bottles. Obviously only the Winkler method approaches natural conditions, because even in stormy weather plankton cells surely are not shaken 100 times a minute as in the Warburg vessels.

Values obtained by the Winkler method for the net (not gross) rate of photosynthesis are somewhat too low. For obtaining the real values of the net photosynthetic rate, determinations of respiration in the dark bottle should probably be carried out in water that is in equilibrium with pure oxygen and not with air.

[1] We are indebted to Dr. O. Kandler for these determinations of *Anabaena* respiration by the Warburg method.

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HYNES, H. B. N. 1958. **A key to the adults and nymphs of British stoneflies (Plecoptera).** British Freshwater Biol. Assoc., Scient. Publ. No. 17. 87 pp. 5s. 11d. (U.S. $0.83).

Changes in this revised edition of Dr. Hynes's 1940 key incorporate up-to-date information on the order in the British Isles, and a more natural arrangement of certain genera into families. The attention to minutiae which has made it possible to recognize practically all British stoneflies in the nymphal as well as the adult condition is a source of envy and embarrassment to North American students. The latter have not yet treated such genera as *Nemoura* (s.l.), *Capnia* and *Alloperla* in anything like so painstaking a fashion—a neglect only partially excused by the more varied fauna with which they deal. The British list now stands at 34 species, including three not recently recorded; known occurrences are illustrated in 30 distribution maps. Hynes's keys to families and, up to a point, to genera, are useful when applied to North American specimens of both adults and nymphs, so that this convenient, well-illustrated, and inexpensive handbook should have a place in every library of aquatic entomology. The systematic arrangement differs from recent American manuals only in that our "sub" categories are avoided—they are either considered full-fledged families and genera, or else are not mentioned.

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ROSS, H. H. 1956. **Evolution and classification of the mountain caddisflies.** University of Illinois Press, Urbana. 213 pp. $6.00.

"Paleozoogeography without fossils" would have been a suitable title for this impressive work. Paleontologists, when they have a good fossil record, are able to trace the origin, the dispersal, and the changes, in space and in time, of groups of animals represented to-day by very few if any species. When fossils are absent, the task of tracing a phylogenetic history on a geographical basis becomes enormously more difficult: for example, who would have guessed that the horse family had most of its development in North America, if he knew only its modern distribution? To study prehistoric zoogeography from living animals special conditions are necessary. The group studied (1) should have a fairly large number of extant species; which (2) must inhabit large sections of most or all continents; (3) many or all species should be restricted to a rather limited habitat, the geographical distribution of which will have shifted with the vicissitudes of geological events; and (4) the world fauna must be reasonably well described. The mountain caddisflies, requiring cool and running water, are admirably qualified in the first three respects, and the present work makes up some lingering deficiencies in respect to No. 4. For tracing the past distribution of living and "reconstructed" types, Dr. Ross relates his detailed knowledge of the structure and relationships of the world caddis fly fauna to a close understanding of geological events. Fossil caddis flies are not completely lacking, of course, but the only thing approaching a "fauna" is restricted to one place and time—the Baltic amber—and it is not too much to say that the same picture would have been obtained if these had not been preserved.

The book contains 370 figures, mostly illustrating details of structure of individual genera and species, of which 4 genera and 37 species are new. More interesting to the non-specialist are the 45 charts showing phylogenetic relationships, particularly the last nine, which have the geological time scale on the ordinate. Conditions permitting intercontinental dispersal of species of a given ecological type (cool-adapted, temperate, or warm-adapted) are found to be rather infrequent and brief, divided by long intervals of separation and endemism. The first intercontinental movement that can be postulated with some assurance is dated in the middle or upper Cretaceous, when cool-to-temperate species moved between Australia, Asia, and the Americas, possibly also Africa. The Laramide revolution of the Paleocene permitted the most extensive interchange of cool-water forms between Asia and America. During the Eocene, by contrast, the cool-water species developed in isolation in the various continents, while there was a remarkable world-wide dispersal of *Chimarra* and other subtropical genera—an event which can apparently be recognized in certain other insect orders. In the middle Tertiary, probably ending in the early Miocene, a dispersal of temperate genera occurred concurrently with the spread of the great deciduous forests. During the Miocene there were recognizable movements of cool-water species from Asia to Europe, and of tropical species from Asia to Australia and Melanesia. The Pliocene and Pleistocene exhibit mainly dispersal within mountain systems. There was however some movement of cool-water forms across the Bering land bridge, and (at least in North America) some movement of mountain species across the plains can be traced.

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Scourfield, D. J., and J. P. Harding. 1958. **A key to the British species of freshwater Cladocera.** 2nd edition. British Freshwater Biological Association, Scient. Publ. 5. 55 pp. 4s. 6d. (= $0.63).

Through the efforts of professional and amateur microscopists, the cladoceran fauna of Great Britain has been well known for more than half a century. The present publication serves admirably as an illustrated key to this fauna, and in addition has brief ecological notes and distribution records in Britain for each species included. Because of the almost cosmopolitan distribution of most species of Cladocera, especially in the Holarctic Realm, this booklet would serve almost equally well for Western Europe, and is even useful for the U. S. However, for the U. S. the key would have to be used with some caution, since the fauna of North America is considerably more extensive than that of Europe, especially in certain chydorid genera such as *Pleuroxus*, *Chydorus*, and *Alonella*.

In organization the second edition of this booklet is much better than the first. The family is determined not by working through some rather obscure characters in a key but by comparing the specimen in hand with line drawings of typical genera.

For each family there is an illustrated key to the species. The ecological and distributional information for each species is given in a short paragraph immediately following the terminal listing of the species in the key. This arrangement is convenient to use and should facilitate identification of cladocera by the non-specialist.

Much of the revision involves a mere rearrangement of the material in the first edition. For the highly variable genera *Daphnia* and *Bosmina*, however, there is a new treatment. In *Daphnia* three groups of species are recognized—the *magna*, *pulex*, and *longispina* groups. For *D. hyalina* and *D. cucullata* a number of varieties are recognized. The revision includes three species of *Daphnia*—*carinata*, *ambigua*, and *curvirostris*—not in the original edition, and these represent the only additions to the fauna.

Bosmina, in accord with current practise, is now separated into just the two species—*B. longirostris* and *B. coregoni*—each with a number of recognizable varieties.

Whereas the nomenclature of the morphologically stable macrothricids and chydorids living in the littoral regions is quite firmly established and satisfactory, that of the highly variable planktonic species is not. The present treatment of *Daphnia* and *Bosmina* is a reflection of current European thinking regarding these two genera.

Harding has done a nice job on this revision. For one who cannot afford Lilljeborg's monograph or cannot locate copies of the works by Keilhack or Wagler, this booklet furnishes good treatment of most of the species of western Europe.

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Brooks, John Langdon. 1957. **The systematics of North American Daphnia.** Mem. Connecticut Acad. Arts Sci., Vol. 13, pages 1–180, incl. 61 plates. Also Yale University Press, New Haven, Conn. Price $8.00.

The impasse in the classification of *Daphnia* which has embarrassed limnologists for decades at long last appears to have been resolved. Brooks' treatment of the genus is exciting, logical, and well documented. It brings the systematics of the North American species into conformity with the current trends in systematics and in the geographical delimitation of species.

Many species of *Daphnia* are highly variable morphologically, both in a geographic and temporal sense. As a result there has been an almost hopeless confusion of names to designate species, subspecies, varieties, forms, etc. Many geographical entities have been designated by trinomials and even quadrinomials. This implied precision of recognition has been negated by the fact, as shown in the present study, that both *Daphnia pulex* and *D. longispina* as formerly recognized is each a composite of many species. This resulted from the separation of these two "species" on the basis of a single character—the presence or absence of a pecten in the middle comb of the postabdominal claw. Convergence has occurred in this character, as indicated by the fact that *D. ambigua* with a "*longispina*-like" claw is related to *D. middendorffiana* which has a "*pulex*-like" claw.

Brooks recognizes 15 species of *Daphnia* from North America. Most of the names used are unfamiliar to limnologists, although no new names are proposed. *D. longispina* described from Europe does not occur in North America. *D. pulex* is retained, although in a much more restricted sense than commonly used hitherto. Morphological variants are not named. The only trinomial used is *D. galeata mendotae*, which is a new combination. This represents the only instance where the North American population of a species is similar to, yet sufficiently distinct from, its Eurasian counterpart to merit recognition. Thus the use of trinomials has been restricted to geographic subspecies in accord with modern practise.

The logic of Brooks' proposed scheme is demonstrated by his fascinating analysis of relationships between species, their phylogeny, recency of origin, etc. Chapter I, "A general account of the systematics of North American Daphnia," is in reality the conclusion of the monograph. The concentration of species in northwestern North

America indicates to Brooks that exchange with Eurasia has occurred across the Bering Strait rather than across the Atlantic, and that western Europe and eastern North America are in reality at opposite ends of the range of distribution. Eight species are holarctic in distribution, and since except for *galeata mendotae* they show the same range of variation in Eurasia and Europe they are not named beyond the species. The other 7 species apparently are of New World origin, although *thorata* and *catawba* may have been derived from stocks still having representatives in Eurasia, and *laevis* is closely related to an undescribed species occurring in Africa south of the Sahara.

One of the most interesting species is *D. middendorffiana*, which is the most successful and often the only species occurring in the Arctic. In this species resting eggs are produced parthenogenetically, which would be of distinct survival advantage where the ice-free season is short and where most of the shallow waters freeze solid in winter. Because of the great morphological uniformity of this species in the Arctic both of North America and Eurasia, Brooks postulates that this whole population may be in reality a single clone.

Other developments of the proposed system of classification which seem to attest to its validity, are the existence of several species pairs, with in each instance the southern member being the more primitive and apparently of greater age.

Without presenting a detailed analysis of species outside the New World, Brooks lists a total of 30 described species for the world as a whole. In 1936 Wagler listed just 4 species in the subgenus *Daphnia*, including the now-discredited *pulex* and *longispina* in the broad sense, whereas Brooks now recognizes 21 species in this subgenus. The number of species in the subgenus *Ctenodaphnia* has been increased only from 7 to 9.

For the North American species there is an illustrated key, followed by a detailed description of the morphology and ecology of each species and its nomenclatorial history. In a couple instances previously unpublished drawings by Richards and Herrick are presented to help establish the validity of the species names used. In a number of instances the original species descriptions are quoted verbatim. Each of the species descriptions is accompanied by large and detailed camera lucida drawings.

One of the valuable parts of the monograph for the average user is Chapter IV, "Regional aspects of *Daphnia* systematics." Brooks recognizes 10 geographical subregions in North America, each of which contains a relatively constant assemblage of species. For a species occupying more than one subregion the intraspecific variation is greater between regions than within a region. For each subregion there is a chart of the species present, with camera lucida drawings of specimens from the subregion, and notes on relative abundance and types of habitat occupied.

With the systematics of North American *Daphnia* now in hand it should be possible to work out some of the phylogenetic problems from subfossil remains in lake sediments. Identification at the present level of our knowledge has to be based entirely on postabdominal claws and the adjoining postabdominal teeth, or on ephippia. Mandibles occur abundantly, and also the filter combs and other thoracic appendages, but these have not been studied sufficiently to enable species recognition from the isolated parts. As examples of the types of information obtainable, Brooks reports the occurrence of *D. catawba*, a southern species, near the beginning of the Cl pollen zone (7000–8000 years ago) in the sediments of a Connecticut pond; and Ogden (unpublished) has found the ephippium of *D. middendorffiana*, an arctic species, in the late-glacial pollen zone A4 from Martha's Vineyard.

The primary goal of systematics is the definition of species. Once this has been achieved, then problems of introgression, interspecific competition, phylogeny, etc. can be dealt with satisfactorily. In the systematics of *Daphnia* Brooks has wrought a mutation that is scholarly, logical, appealing, and unquestionably has survival value.

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